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Contents

Preface

*This book covers key areas of Medicine and Medical Research. The contributions by the authors include CD28 response element, Staphylococcal Enterotoxin A-E, Extracellular signal regulated kinase, c-Jun N-terminal kinase, Perioperative and postoperative events, patient satisfaction, Mephentermine, anxiety, Abdominoplasty, muscle-aponeurotic plication, plication, lipoabdominoplasty, breech delivery, caesarean section, external cephalic version, planned vaginal breech birth, Vertebral artery, variations, vascular surgeons, Transversus abdominis, lumbar lordosis, flexiruler, flexicurve, pressure biofeedbackunit, young adults, age, lumbar stability, floor sitting, ground posture, squatting, cross-leg sitting, lower limb, Asian population, Indian population, range of motion, polycyclic aromatic hydrocarbons, OH-PAH metabolites in urine, psychological tests, cognitive development, bender visual motor gestalt test, raven colored progressive matrices, internal martial art, moving meditation, alternative medicine, anti-aging solution, hyperthyroidism, Hypothyroidism, Toxicosis, Non –Thyroidal, Hormonal changes, SARS-CoV-2, COVID-19, clinical data, FNAC salivary gland, diagnostic accuracy, tumors and compensatory capacity kidneys, caries management, dental caries, pediatric dentistry, silver diamine fluoride, silver fluoride bullet, emotional intelligence, stress tolerance, diabetic physical exercising and diabetic nonphysical exercising peoples, interdependence, skin malignancies, non-Hodgkin lymphoma, uterine, abnormal vaginal bleeding, extra-nodal lymphoma, liver fibrosis score, hypertension, dyslipidemia, diabetes mellitus, metformin, non-alcoholic fatty liver disease, l*ymphoblastic Leukemia. T*his book contains various materials suitable for students, researchers and academicians in the field of Medicine and Medical Research.*

Chapter 1
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The Role of JNK, ERK and p38 Mitogen Activated Protein Kinases in the Response of Jurkat T Cells as a Model for T Cell Activation

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ABSTRACT

Activation of T lymphocytes requires at least two distinct signals for full cellular response. Signal one involves the engagement of the T cell receptor (TCR) by antigen specific peptide-MHC complexes. The signal two is provided by the interaction of costimulatory molecules on the antigen presenting cells (APC) and the corresponding counter receptors on the T cells. In this way, to mimic the two-signal requirements for T cell activation mediated by ligands, we exposed the superantigens SEA or SEE (signal 1) to T cells incubated with HLA-DR/LFA-3 or HLA-DR/B7-1-CHO transfected cells (signal 2). LFA-3 costimulation was able to induce T cell proliferation as well as IFN-γ and IL-4 production at similar levels as in cells induced by B7-1. Analysis of the CD28RE of the IL-2 promoter showed specific transcription factor recruitment at the CD28RE element upon induction by B7 1/SEE. Further functional studies with an IL-2 enhancer-promoter carrying either wild type or mutated versions of the CD28RE site revealed that this element is necessary for full activation upon B7-1 costimulation. While both CD28/B7-1 and CD2/LFA-3 costimulation resulted in the up-regulation of IL-4 and IFN-γ promoters, IL-2 promoter activity and production of IL-2 were only seen after B7-1 costimulation. However, contrary to what has been previously proposed, we show that costimulation with either B7-1 or LFA-3 further enhanced the ERK-2 activity and strongly activated the p38 MAPK pathway, but only B7-1 costimulation induced high levels of JNK-1 activity. These data suggest that the differential effect of CD28 vs. CD2 can be related to the difference in the ability of the two pathways to induce JNK-1 activity.

Keywords: CD28 response element; Staphylococcal Enterotoxin A-E; Extracellular signal regulated kinase; c-Jun N-terminal kinase; Interleukin-2.

1. INTRODUCTION

In addition to T cell receptor (TCR) cross- linking (signal one), optimal T cell activation requires engagement of the costimulatory molecule CD28 by its ligand B7-1 (CD80) on Antigen presenting cells (APC) (signal two) [1,2,3,4,5]. The ligand for CD28 is B7 (B7-1/CD80 and B7-2/CD86), which displays a restricted pattern of expression on APCs, including activated B cells, suggesting an important role for CD28 in the interaction between T and B cells [6,7,8,9]. Furthermore, CD28 costimulation synergizes with TCR signals to increase IL-2 production and T-cell proliferation in a cyclosporine A-insensitive manner [10,11].

Another receptor on T cells that can induce the secondary stimulus required for T cell activation is the CD2 receptor, which recognizes and binds its ligand on the surface of the antigen presenting cells [12,13,14]. Like CD28, CD2 has a relatively large and highly conserved cytoplasmic domain, which allows association with the Src-like kinases, Fyn and Lck [15,16]. Despite that CD2 ligation may also transduce an independent signal, initiating T cell activation [17], it seems that a major role for CD2

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molecules is to enhance the TCR/CD3-or CD28 mediated response, particularly in resting and naive T cells [13,17]. LFA-3, the natural human ligand for CD2, is a glycoprotein expressed on virtually all nucleated cells. It is also a member of the Ig super gene family. Several lines of evidence have demonstrated that CD2 binds to its ligand LFA-3 with high affinity and thus should play a significant role in stabilizing initial cell-cell interactions and adhesion prior to the TCR/MHC class II+complex interaction [18,19,20].

The induction of signals one and two in T cells leads to the activation of several signal transduction pathways, including the MAP kinase family of enzymes [21,22]. These enzymes play a critical role in a diverse number of signal transduction pathways in response to mitogenic stimuli, and they are directly implicated in the linking of cytoplasmic signaling cascades to the control of transcription in the nucleus [23]. The MAPK family is divided into two main groups. The Extra cellularly Regulated Kinases (ERK group) [24] and the Stress- Activated Protein kinase (SAPK group), which includes the c-Jun N-terminal Kinase (JNK) and p38 kinase [25,26]. Dual phosphorylation of these kinases on a TXY motif in their activation loop results in an open, catalytically active, conformation. The active kinases phosphorylate several transcription factors, including Elk-1, cJun, and ATF-2, which are involved in the regulation of c-fos and c-jun gene expression [27,28,29,30].

Several reports using other models, have shown the importance of the MAP kinases for cell proliferation [31]. The activation of c-Jun and c-Fos proteins form a complex called “Activator protein-1” (AP-1) which was first identified as a transcriptional factor that binds to an essential cis element of the human metallothionine IIa (hMTIIa) promoter [32,27,33,34]. Later the binding site for AP-1 was also recognized as the TPA response element (TRE) of several cellular and viral genes including human collagenase, SV40 and IL-2. Indeed, the AP1 complex plays a critical role in controlling IL-2 gene transcription [35,36,37], by binding to three sites: the NF-AT, Oct-1, and AP-1 itself. The expression of the IL-2 gene is regulated by its 5´- flanking promoter sequence and its critical regulatory and inducible activity is contained within the 300-bp region immediately upstream of the start site [38,39,40,41]. The IL-2 promoter includes defined binding sites for the transcription factors NF-AT, NF-κB, AP-1, Oct-1, and CD28RC [42,43,44]. Thus, activated T cells produce IL-2, express high affinity IL-2 receptors, and proliferate in an autocrine or paracrine fashion. However, the interaction of the CD28 and B7-1 molecules has been recognized as the major pathway in the regulation of IL-2 expression, and subsequently several reports have demonstrated its specificity to this respect [38,45,46]. Early reports showed that CD3 cross-linking by antibodies could induce ERK-2 activity and that antibodies against CD28 failed to increase the activity of ERK-2 in Jurkat T cells [26,37]. However, one major discrepancy of this study compared with others was that we could strongly increase the ERK-2 activity after TCR and LFA-3 or CD28 costimulation. This discrepancy may reflect differences in the use of CD3 and CD28 antibodies v/s a superantigen and B7-1 costimulatory molecule expressed in CHO-DR cells. A role for the JNK and ERK cascades in T cell activation has been described, but the links between receptor engagement and downstream events still need to be defined.

2. MATERIALS AND METHODS

2.1 Reagents

Staphylococcal enterotoxins A and E (SEA, SEE) were purchased from Toxin Technology (Madsion, W I). Ficollisopaque, G418 and L-methionine sulfoximine (MSX) were purchased from Pharmacia Inc. (Uppsala, Sweden). The protease inhibitors phenylmethylsulfonyl fluoride (PMSF), leupeptin, pepstatin, aprotinin and bestatin were from Roche, USA. [γ-32 P]ATP was from Amersham. T4 polynucleotide kinase and poly (dI-dC), were obtained from Amersham Pharmacia Biotech (Piscataway, NJ). Tris Borate EDTA buffer and acrylamide-bisacrylamide (29:1) were obtained from Bio-Rad (Richmond, CA). PHAS-1 was purchase from Strategene (La Jolla, CA). Luciferase assay reagent, lysis buffer and the pGL-2 luciferase vector were obtained from Promega (Madison, WI). Recombinants and mAbs to human IL-2 (5344.111 and B33-2), IL-4 (8D4-8, MP4-25D2) and IFN-γ (NIB42, 4S.B3) were obtained from PharMingen (San Diego, CA). Phorbol-12Myristate-13-Acetate (PMA) and Ionomycin were purchased from ICN Pharmaceuticals (Costa Mesa, CA). GSTcJun, GST-ATF-2 were a gift from Dr. Roger J. Davis (Howard Hughes Medical Institute, MA). Production and

purification of GST-cJun and GST-ATF-2 proteins were performed as described [25]. Cell separation, culture and stimulation Peripheral blood lymphocyte (PBL) were isolated from buffy coats obtained from healthy blood donors by density centrifugation over Ficoll-Paque (Pharmacia Biotech, Uppsala Sweden) as previously described [47]. T cells were enriched by separation over a gelatin column followed by positive selection of CD4+ T cells by MACS (Miltenyi Biotech, Sunnyvale, CA) according to the manufacturer's description. The purity of the separated cells was routinely checked by FACS analyses and was 99% CD4+ cells.

The purified CD4+ T cells were resuspended in RPMI 1640 supplemented with 2 mM glutamine and 10% FCS. The cells were stimulated with 10 ng/ml SEA presented by CHO transfectants at a T:CHO ratio of 20:1. After 72 h, the cultures were pulsed with 0.5 µCi[3H] thymidine and harvested after an additional 4 h. The amount of incorporated [3H] thymidine was determined by liquid scintillation counting. The human T leukemia cell line Jurkat was maintained at logarithmic growth in RPMI 1640 supplemented with 2 mM glutamine and 10% fetal calf serum. The transfected CHO cells were maintained in the same medium with G418 and/or MSX. Stimulation of the T cells with SEE was done at a concentration of $1x10^6$ cells/ml in the presence of $0.1x10^6$ cells/ml CHO cell transfectants at 37 °C as previously described [1,47]. Transfected cell lines CHO cells stably transfected with the cDNAs encoding the human HLA-DR, B7-1 and LFA-3 cell surface molecules have been described in detail elsewhere [47]. Single and double transfectants expressing similar levels of the transfected molecules were established by repeated cell sorting and they were periodically reanalyzed.

2.2 Plasmids

The human IL-2 promoter-enhancer fragment [40,41], nucleotides -500 to +60, was subcloned from pSV-IL-2 CAT into the luciferase reporter vector pGL2 (Promega, Madison, WI). The -500-to +60 fragment was prepared by PCR with appropriate primers creating a 5´- XhoI site and a 3´-HindIII restriction sites. The IL-2 promoter mutated in the CD28RE region was generated by PCR directed splicing overlap extension, to replace positions -159 to -164 with the sequence 5´-CCTCGA-3´. The constructs were confirmed by sequencing.

2.3 Preparation of Nuclear Extracts

Human Jurkat leukemia CD4 T cells (3-5 $x10^7$ cells) were stimulated with various irradiated transfected CHO cells (8000 rad) in the absence or presence of SEE (100 ng/ ml) at 37°C (in a humidified atmosphere containing 5% CO_2). The cells were harvested after 6 hr of culture and T cells were separated from CHO cells using a plastic adherence technique. The harvested T cells were re-suspended in 10 ml PBS and pelleted by centrifugation for 5 min at 1500 rpm. The pellet was resuspended in 1 ml PBS, transferred into an Eppendorf tube and repelleted by spinning for 15 sec in a microfuge. PBS was removed and the cell pellet was re-suspended in 500 µl cold buffer A (10 mM Hepes pH 7.8; 15 mM KCl; MgCl2 2mM; 0.1 mM EDTA; 1mM DTT; 1 mM PMSF). T cells were allowed to swell on ice for 15 min, 25 µl of a 10% solution of NP-40 was added, and the tube was vortexed vigorously for 10 sec. The homogenate was centrifuged, and the nuclear pellet was re-suspended in 100 µl ice-cold buffer B (20 mM HEPES pH 7.9; 0.4 M NaCl; 1mM EDTA; 1mM EGTA; 1mM DTT; 1 mM PMSF) and the tube was rocked for 15 min at 4°C on a shaking platform. The nuclear extract was centrifuged for 5 min at 4°C and the supernatant was frozen in aliquots at -70°C. Before use of the buffers A and C, a mixture of the protease inhibitors was added: 0.5 µg Leupeptin/ml; 0.7 µg Pepstatin/ml; 1µg Aprotinin/ml and 40 µg Bestatin/ml. 1-2 µl of the nuclear extract (2-4 µg protein) was used for a gel shift assay in the presence of 3 µg poly dI-dC as previously described [42].

2.4 Electrophoretic Mobility Shift Assay (EMSA)

The double-stranded oligonucleotides corresponding to the wild type, mutated CD28RE of the IL-2 promoter were (coding strand): 5´-CTCAAGATCGAAATTCCAAAGAGAC-3´, and 5´-CTCAAGATCGACCTCGAAAAGAGAC-3´, respectively (the mutation is underlined). One to 2 µl of nuclear extract corresponding to 5-10 µg of protein were added to 4 µl binding buffer containing 2 to 3 µg poly (dI-dC)2 as a non-specific competitor. The reaction mixtures were incubated at 37°C for 30

min with 15,000 cpm of double stranded P labeled oligonucleotides in a final volume of 15 µl. The samples were electrophoresed on 5% polyacrylamide gels in 89 mM Tris,89mM boric acid, 2 mM EDTA. The gels were fixed in 40% methanol and 10% acetic acid for 15 min, dried, and visualized by autoradiography [42]. DNA transfection and Luciferase activity assay.

Transfection of Jurkat cells was carried out by electroporation. Briefly, plasmid DNA was mixed with exponentially growing Jurkat cells (20 x 10^6 cells/ml) in complete medium and the cells were electroporated in an electro cell manipulator 600 (BTX, San Diego, CA.) using 130V/1700 µF capacitance. The transfected cells were cultured for 24 hrs before being stimulated with the different CHO-transfected cell lines with or without 100 ng SEE/ml. After various periods of time, cells from each independent well were harvested, washed twice in PBS and treated with lysis buffer (Luciferase Assay Promega, Madison, WI) for 5-10 min on ice. Lysates were spun down for 1 min and the total supernatants were analyzed using Luciferase Reagent (Promega) and measured as a duplicate in a luminometer (MicroLumat LB 96 P, Berthold) for 5 s. Background measurement was subtracted from each duplicate and experimental values are expressed either as recorded light units, Luciferase activity or as relative activity compared to extracts from unstimulated cells. JNK/ERK/p38 Kinase Activity Assays Jurkat T cells were incubated with or without SEE and the different CHO cells (CHO-DR, CHO-DR/B7-1 and CHO-DRLFA-3) for 30, 60, and 120 minutes (for ERK-2 and JNK-1) or 60, 120, and 180 min (for p38). After time periods the CHO cells were removed by plastic adherence and the Jurkat T cells were collected and lysed, and the proteins of interest were immune precipitated using mAb to JNK-1 (Santa Cruz), ERK-2 and p38 and protein A sepharose CL-4B beads for 3 to 4 h. Beads were washed 3 times with lysis buffer, twice with LiCi and twice with kinase buffer (25 mM HEPES, pH 7.4, 25 mM glycerophosphate, 25 mM MgCl, 25 mM DTT, and 0.1mM Na3VO42), and finally resuspended in 30 µl of kinase buffer. The beads were then incubated with human GST-c-Jun (1-79) for JNK-1, GST-ATF-2 (1-109) for p38, PHAS-I (Strategene, La Jolla, CA) for ERK-2, and 1 µl of [γ -32 P]ATP at 30°C for 30 min. One volume of 2x sample buffer (125 mM Tris, 6% SDS, 20% glycerol) was added, and the reaction mixtures were boiled at 100°C for 3 min. Phosphorylated proteins were analyzed by SDS-PAGE and autoradiography. Cytokine assays Cytokine levels in supernatants of unstimulated and stimulated T cells were determined by specific ELISAs using a sandwich technique as previously described [48] and according to the manufacturer. Recombinant cytokines used for standard curves in ELISA and specific Ab pairs for each cytokine were obtained from PharMingen (San Diego, CA); IL-2 (5344.111 and B33-2), IL-4 (8D4-8, MP425D2) and IFN-γ (NIB42, 4S.B3).

3. RESULTS

B7-1 and LFA-3 costimulation in Jurkat cells induce distinct protein binding complexes that recognize the CD28RE sequence contained in the IL-2 promoter. We first investigated the relative role of B7-1 and LFA-3 on the transcriptional regulation of the IL-2 promoter. The minimal inducible IL-2 enhancer region has been identified as a 300 bp region upstream of the transcription start site. Several target sites recognized by various transcription factors have been identified within this region, including the CD28RE, AP-1, NF-AT and NF-κ B [43,38]. However, mutational analyses have demonstrated that the CD28RE plays a crucial role in the transcriptional activation of the IL-2 promoter.

Electrophoretic mobility shift assay (EMSA) was used with nuclear extracts prepared from costimulated Jurkat T cells and oligonucleotides encoding the wild type radiolabeled CD28RE of the IL-2 promoter to analyze the protein complexes that recognize this sequence. Fig. 1 shows that costimulation with SEE and B7-1 or LFA-3 induces distinct nuclear factor or binding protein complexes which strongly bind to the CD28RE sequence (Fig. 1A).

Unstimulated and HLA-DR stimulated Jurkat cells did not contain detectable levels of nuclear factors (Fig. 1A). No binding was detected in Jurkat cells presented with HLA-DR either treated or not with SEE. However, when cells were costimulated with B7-1 or LFA-3, strong protein binding complexes were detected, which were differently activated by both receptor pathways. These protein-DNA complexes were not competed with cold Oct-1 specific oligonucleotide suggesting that present in these complexes. As a control, we stimulated Jurkat cells (not presented with CHO transfectants) with SEE or PMA/ Ionomycin. As observed with the CHO transfectants, SEE alone did not induce any

specific protein binding complex. However, PMA/I induced a strong complex with a higher electrophoretic mobility than that observed with B7-1/LFA-3 costimulation.

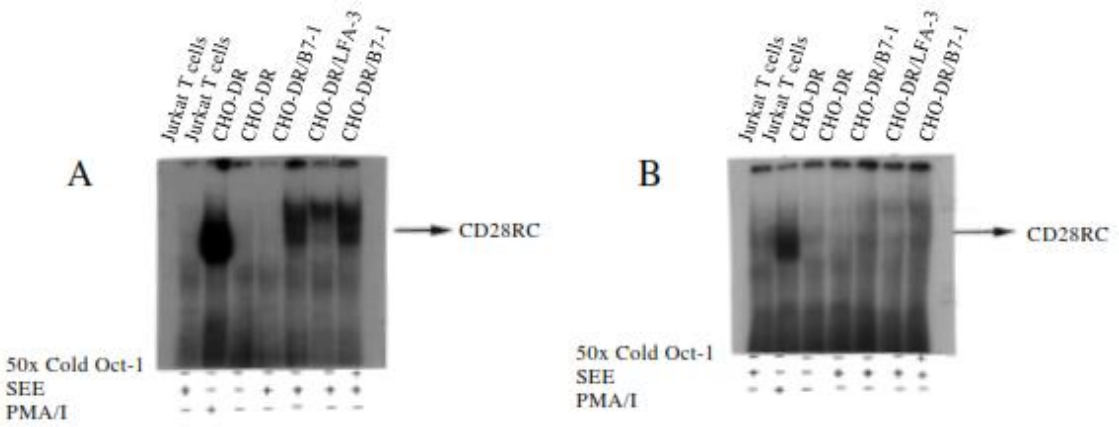

Fig. 1.

Jurkat T cells were incubated in RPMI supplemented with 10% FBS. The cells were the stimulated with the various indicated CHO transfectants for 6 h, in the presence or absence of SEE 100 ng/ml, or with PMA/Ionomycin as a positive control. Nuclear cell extracts were prepared and analyzed by EMSA. In A, protein binding to the CD28RE of the IL2 promoter using a oligonucleotide corresponding to the 5´-CTCAAGATCGAAATTCCAAAGAGAC-3´, of the IL-2 promoter. In B, the mutated version of the IL-2 promoter sequence showed above. 5´-CTCAAGATCGACCTCGAAAAGAGAC-3´. Sequence specificity of the CD28RE was assessed by incubating nuclear extract with radiolabeled CD28RE oligonucleotides in the presence of no competitor Oct-1 at 50-fold molar excess (5´CGTCTCATGCGATGCAAA-TCACTTGAGATC 3´). The mutation is showed in bold face. The results from one of two similar experiments are shown.

When the same Extracts were incubated with a mutated CD28RE sequence, the DNA-protein binding activity was completely abolished (Fig. 1B). Only a residual binding activity was observed in Jurkat cells stimulated with PMA/Ionomycin.

Strong costimulatory effect of B7-1 and LFA-3 on transcriptional induction of IL-2 promoter activity by B7-1 and LFA-3 required a functionally intact CD28RE. The functional importance of the CD28 response element was further dissected comparing the transcriptional response of transiently transfected Jurkat T cells with a luciferase reporter-construct carrying the wild type IL-2 and the mutated version of this CD28RE. As we previously reported [47], a strong induction of the IL-2 promoter activity was observed by costimulation of Jurkat T cells with SEE and B7-1, but not with SEE and LFA-3 (Fig. 2).

Functional demonstration of the specificity of the CD28RE in the transcriptional activity of a wild type and a mutated version of the IL-2 promoter. Jurkat cells were transfected with the wild type of IL-2 luciferase reporter gene or with the mutant version. After 24 hours incubation the cells were stimulated with SEE and the CHO and CHO transfectants CHO-DR, CHO-DR/LFA-3 and CHODR/B7 1. Eight hours later, samples were harvested and analyzed for luciferase activity. The native CD28RE sequence of the IL-2 promoter promoter 5´ - AAATTC-3´ was mutated to the 5´-CCTCGA - 3´ sequence (mutation shown in bold-face). Luciferase activity is expressed as arbitrary light units minus background units of buffer alone. Results from one of two similar experiments are shown.

Importantly, this activity was severely reduced in the IL-2 promoter carrying a mutation in the CD28RE. We showed that the transcriptional activity of an IL 2 promoter reporter construct carrying a mutated version of the CD28RE was strongly reduced in B7-1 costimulated Jurkat T cells compared to the wild type (Fig. 2).

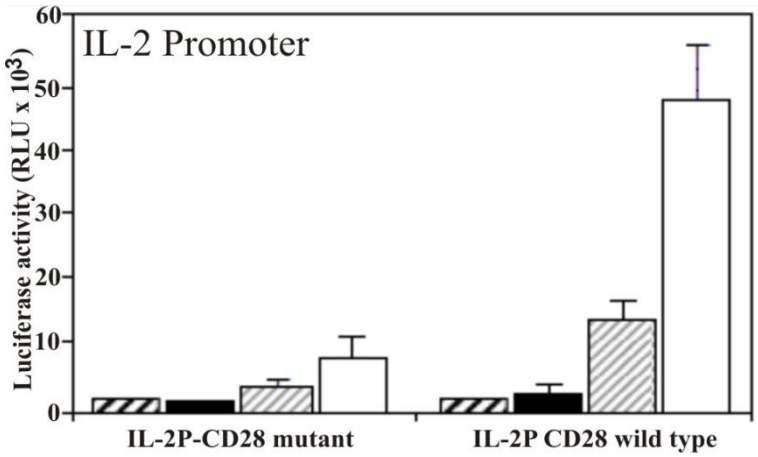

Fig. 2.

These results further support a major role of the CD28/B7-1 pathway in the induction of IL-2 and T cell activation. Requirement of both signals for an optimal induction of JNK-1, ERK-2, and p38 kinases activities. To examine whether SEE (signal one) alone or together with LFA-3 or B7-1 (signals two) costimulation can activate the mammalian JNK-1, ERK-2 and p38 kinase cascades, we activated Jurkat T cells with and without SEE and costimulated with CHO-DR, CHO-DR/B7-1, or CHO-DRLFA-3 for given periods of time. JNK-1 was immunoprecipitated from the lysates (using a specific antibody, JNK1 (C17): sc-474, Santa Cruz Biotechnology, Inc.) kinase assay with GST-c-Jun, (amino acids 1-79) as protein substrate (Fig. 3). We showed that optimal induction of JNK-1 activity required the participation of both signals, since SEE alone did not affect basal levels of the kinase activity. Substantial activation of JNK-1 was already observed after 30 min of costimulation. This activity peaked around 60 min after B7-1 costimulation and decreased after 120 min (Fig. 3C).

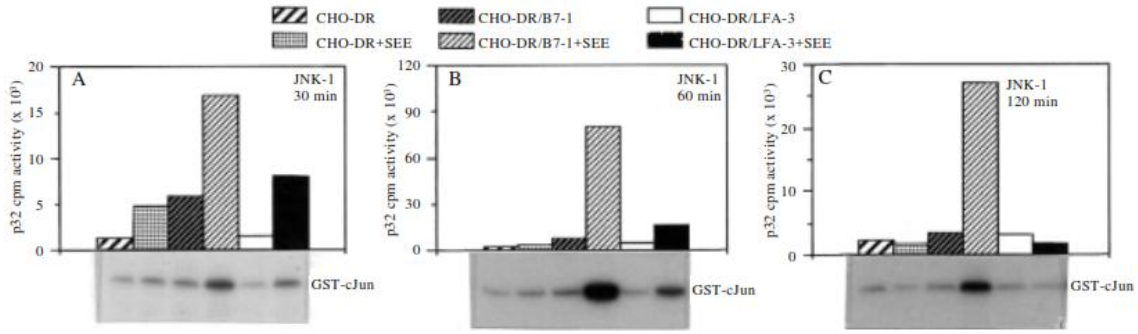

Fig. 3.

Activation of the JNK-1 MAP kinase member by SEE (100 ng/ml) and the CHO-DR, CHO-DR/B71 and CHO-DR/LFA-3. Jurkat cells were incubating in medium containing 10% FBS. The cells were stimulated with and without SEE and the various CHO transfectants for the indicated periods of time (in minutes). Whole cell extracts were prepared and assayed for JNK-1 using and immunocomplex kinase assay with GST-c-Jun as substrate. The cpm activity with respect to untreated cells is shown above the kinase assay panel.

To determine whether SEE (signal one) and LFA-3 (signal two) costimulation could also activate the mammalian ERK-2 and p38 pathways, similar assays were employed. ERK 2 and p38 were immunoprecipitated and their activities were determined by phosphorylation of either PHAS-1 protein or GST-ATF-2 respectively. As reported previously, ERK- 2 activity was elevated 30 min of costimulation with signal one (CHO DR/SEE) (Fig. 4A). However, in our system, LFA-3 and B7-1 costimulation (signal two) increased the activity of ERK-2 several fold, suggesting a principal role for

signal two in the up-regulation of ERK-2 activity. The optimal activity peaked 60 min after DR, DR/B7-1 and DR-LFA-3 costimulation (Fig. 4B) and decreased after 120 min (Fig. 4C). Interestingly, LFA-3 costimulation proved to be more effective than B7-1 in increasing the levels of ERK-2 activity (Fig. 4B and C).

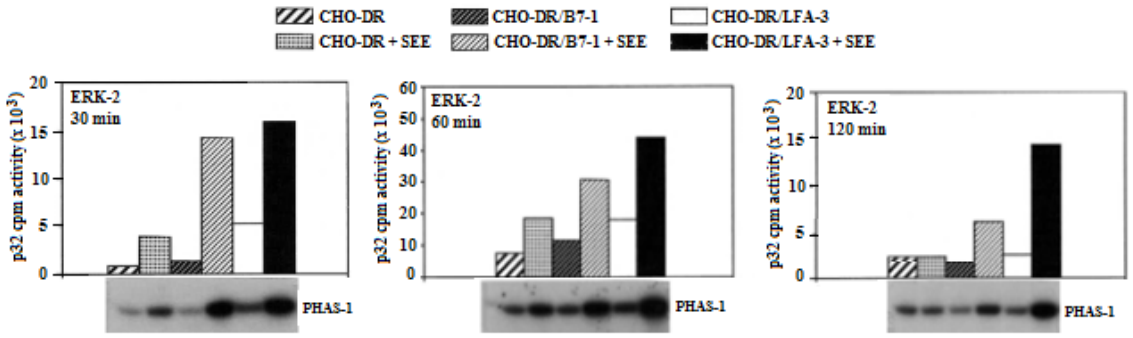

Fig. 4.

Activation of the ERK-2 MAP kinase member by SEE (100 ng/ml) and the CHO-DR, CHO-DR/B71 and CHO-DR/LFA-3 in Jurkat T cells stimulated with and without SEE and the various CHO transfectants for the indicated periods of time (in minutes). Whole cell extracts were prepared and assayed for ERK-2 using and immune complex kinase assay with PHAS as substrate. The cpm activity with respect to untreated cells is shown above the kinase assay panel. The experiments were performed at least twice with identical results.

These results suggest a synergistic role between TCR and CD2 to superinduce the levels of ERK-2 activity. We finally investigated the activation of p38 kinase. Like JNK-1, B7-1 costimulation strongly activated p38 kinase activity in Jurkat T cells (Fig. 5). No p38 kinase activity was detected in the absence of costimulation, suggesting the requirement of both signals to induce p38 activity. However, activation of p38 kinase activity was only observed after 120 min of costimulation with either B7-1 and or LFA-3 and decreased to basal levels after 180 min of costimulation (Fig. 5).

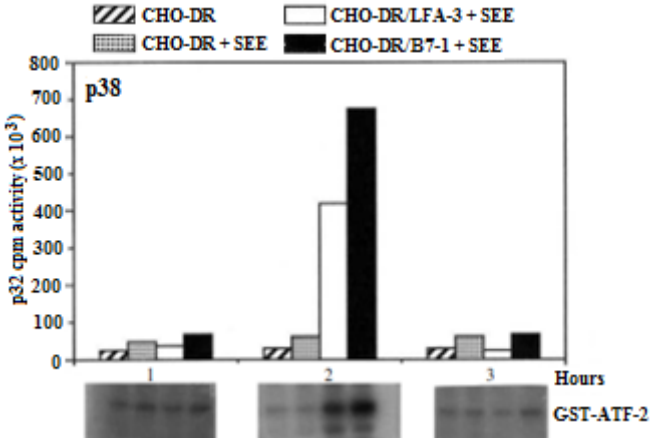

Fig. 5.

Activation of the p38 MAP kinase member by SEE (100 ng/ml) and the CHO-DR, CHO-DR/B7-1, CHO-DR/LFA-3 and Jurkat cells were incubating in medium containing 10% FBS. The cells were stimulated with and without SEE and the various CHO transfectants for the indicated periods of time (in minutes). Whole cell extracts were prepared and assayed for p38 using and immune complex kinase assay with GST-ATF-2 as substrate. The cpm activity with respect to untreated cells is shown above the kinase assay panel.

Western blot analysis confirmed that JNK-1, ERK-2 and p38 kinases were expressed at comparable levels (data not shown).

Costimulation by B7-1 strongly induces the transcriptional activity of the IL-2, INF-γ and IL-4 promoters-enhancer region in Jurkat T cells transiently transfected with luciferase reporter constructs. Based on the role of the CD28/B7-1 pathway in T cell activation, we wanted to analyze the effects of LFA-3 and B7-1 costimulation on the transcriptional activity of several promoters that play important roles in the immunological response, such as IL-2, IFN-γ and IL-4. Jurkat T cells were transiently transfected (electroporation) with the human IL-2 (Fig. 6A) IFN-γ (Fig. 6B), and IL-4 (Fig. 6C) promoter regions fused to the luciferase reporter gene. The transfected cells were then stimulated with SEE (100 ng/ml) and presented to the different CHO transfectants, after which the luciferase activity was measured. As shown in figure 6, stimulation with SEE alone in the absence of signal two did not affect the transcriptional activity of the different promoters (Fig. 6 A, B, and C). Similarly, signal two (B7-1 or LFA-3) had no effect in the absence of SEE. However, costimulation with B7-1 resulted in a very strong transcriptional activation of all three promoters, IL-2, IFN-γ, and IL-4 (Fig. 6 A, B, and C). In contrast, costimulation with LFA-3 had no effect on IL-2 transcriptional activity, but it produced a strong activation of both IFN-γ (Fig. 6B) and IL-4 (Fig. 6C) promoter.

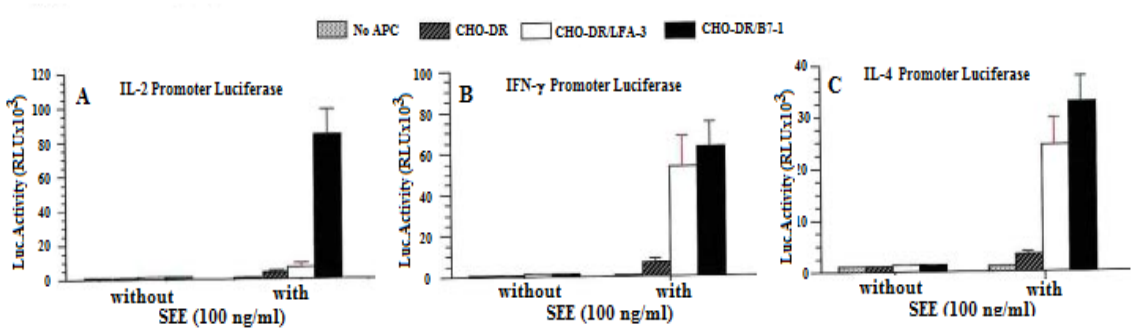

Fig. 6.

Comparison of the transcriptional regulation of the IL-2, IFN-γ and IL-4 promoters in Jurkat T cells costimulated with CHO-DR, CHO-DR/LFA-3 and CHO-DR/B7-1 transfected cells. Jurkat T cells were transfected with the various luciferase promoter reporter genes and cultured in RPMI supplemented with 10% FCS. After 24 h the cells were treated with SEE and cultured for 8 h with the various CHO transfectants (1:10 ratio to T cells). Luciferase activity is expressed as RLU minus background units of buffer. The results shown are mean values from three similar experiments. Error bars indicate standard errors of the mean.

Proliferative effect of costimulation on proliferation response and IL2 production of CD4+ T cells to SEA and CHO-DR, CHODR/LFA-3 and CHO-DR/B7-1 Finally, we investigated the effect of B7-1 or LFA-3 costimulation on the induction of proliferation and the production of IL-2, IFN-γ and IL-4 and correlated these results with the transcriptional activity of the MAP kinases ERK-2, JNK-1 and p38. T cell proliferation and IL-2, IFN-γ, and IL-4 production were measured in freshly prepared CD4+ T cells ($1x10^6$/ml) cocultured with 10 ng/ml SEA and with either CHO-DR, CHO-DR/LFA-3, or CHO-DR/B7-1 for 72 h at 37°C. (Fig. 7). B7-1 costimulation was required to induce large amounts of IL-2 (Fig. 7A) while both B7-1 and LFA-3 costimulation induced large amounts of IFN-γ (Fig.7B,C).

However, only marginal levels of IL-4 were detected in supernatants of T cells costimulated with B7-1 and LFA-3. The proliferation activity that clearly correlated with the induction of IL-2 and IFN-γ, also required B7-1 costimulation (Fig. 7D).

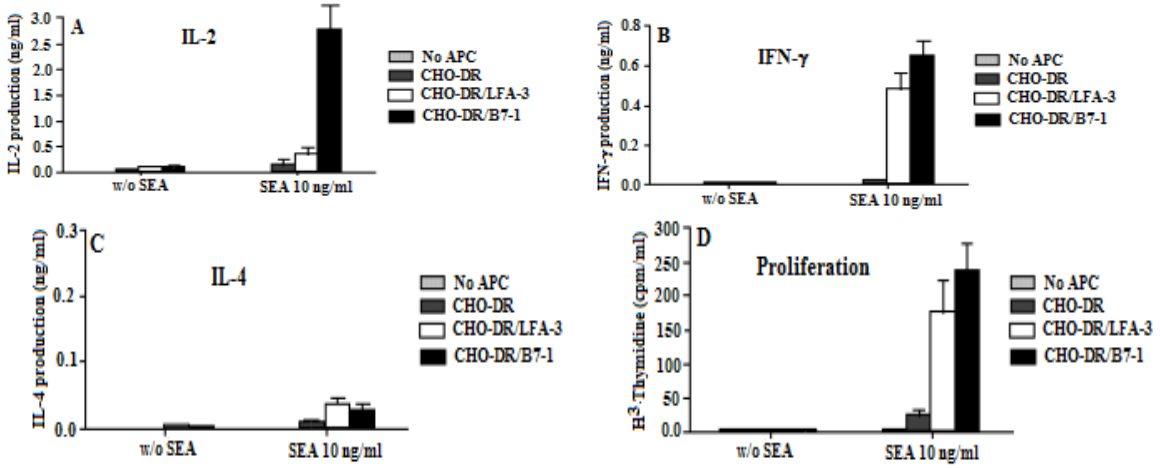

Fig. 7.

Costimulatory effect of LFA-3 and B7-1 in induction of IL-2 (A), IFN-γ (B) and IL-4 (C) production and proliferation (D) of SEA primed T cells. In A, CD4 + T cells (1x106 /ml) were cultured for 3 days with RPMI supplemented with 10% FBS, CHO-DR, CHO DR/LFA-3 and CHO-DR/B7-1 (0.1x10 6/ml) with or without SEA (10 ng/ml). In A, B, and C, supernatants from the same culture were collected on day 2 and assayed by ELISA for the production of the above mentioned citokines. In D, the [3 H]TdR incorporation for proliferation was assessed the last 4 h of culture. Experiments were done at least three times and the most representative results were shown. Error bars indicate standard errors of the mean.

4. DISCUSSION

One of the most interesting signaling pathways involved in the activation of T cells is represented by the MAP kinase cascade. Signal one activates ERK-2 pathway and is involved in the survival of T cells [26,21,23]. The second signal delivered via accessory molecules such as CD28 and CD2 led to the activation of the JNK and p38 pathways, responsible for induction of IL-2 receptor expression, IL-2 production and subsequent clonal expansion. Contrary to what has been previously proposed [26,49,23] we show that the requirement for at least two signals for the efficient activation of T cells is not unique to JNK, as the ERK is also fully activated by TCR engagement and B71 or LFA-3 costimulation. We showed by kinase assays that T cells activated with SEE (signal one) alone substantially activated ERK, but not JNK or p38 pathways.

On the other hand, T cells activated with SEE and costimulated with CD28 or CD2 (signal two) showed a strong activation of all three pathways (ERK, JNK and p38). Our results also contrast with recent studies showing that the CD28 was not required for JNK activation in normal murine T cells [50]. In this study high concentrations of anti CD3 mAb alone were sufficient for ERK and JNK activation, even in the absence of anti CD28. This discrepancy may reflect differences in the use of superantigens SEE (signal one) and CHO transfectants expressing the ligands for CD28 and CD2 (signal two) in our studies, as compared with the use of anti-CD3 and anti-CD28 antibodies to induce a similar response or our use of a more physiological system for activation of T cells. The MAP kinases regulate the activity of AP-1, a nuclear factor that plays a critical role in IL-2 gene transcription by binding to three different sites; NF-AT, Oct-1, and AP 1 itself [40,41]. It has also been demonstrated that JNK activates the c-fos promoter through phosphorylation of TCF/Elk-1 [27] and that MEKK-1 kinase, an upstream kinase in the cascade of JNK, can also induce c-fos and Elk-1 transcriptional activity in the presence of lower levels of ERK [26,28], suggesting the involvement of mechanisms other than the ERK signal in the activation of c-fos. In other systems, JNK, unlike ERK, is not activated by the phorbol ester alone, but rather requires the phorbol ester in combination with calcium ionophore [21,49,23]. Triggering of CD3 and CD28 also results in CsA- sensitive activation of JNK, whereas each stimulus alone results in little or no activation. However, the dramatic reduction in

transcriptional IL-2 promoter activity observed with a luciferase reporter driven by IL-2 promoter containing a mutated CD28RE further underscores the functional importance of the B7-1 costimulation for IL-2 transcriptional activity. These observations, along with the EMSA experiments, indicate the presence of a functional CD28RE in the IL-2 promoter. It is worth commenting that LFA-3, in contrast with B7-1, cannot costimulate IL-2 production, but can costimulate cell proliferation. The results suggest that the differential effect of CD2/LFA-3 vs. CD28/ B7-1 on IL-2 regulation could be related to the difference of the ability of the two pathways to induce JNK activity. In this regard, other laboratories have reported that the activation of ERK, JNK and p38 is differentially sensitive to inhibitions of PI3K [51]. Both CD2 and CD28 are linked to the PI3K pathways, suggesting an alternative mechanism of activation of JNK and thus regulation of IL2 gene expression. The adhesion of the complex induced by the CD2/LFA-3 pathway was also inhibited by the loss o the functional CD28RE. However, earlier reports indicated a central role of CD28RE in CD28-costimulated T cells, although several studies suggested that the CD28RC was not exclusively seen in CD28stimulated T cells [52,53]. Using native B7-1 and LFA-3 ligands, we found that the CD28RC is detectable in EMSA by both B7-1 and LFA-3 costimulation. However, the B7-1 costimulation forms two distinct bands in the gel shift assay while the LFA-3 costimulation induces only the upper band.

We speculate that the complex bound to the CD28RE, corresponding to the faster migrating band, may be involved in activation of IL2 promoter, as it is induced only by B7-1 and binds to CD28RE on its own as well as in combination with nuclear factors, such as AP-1. In our system, elevated ERK and JNK activities can be detected 1 h after SEE presentation to T cells, while p38 kinase induction was observed only after 2 h, particularly in the presence of B7-1 costimulation. Thus, early activation of these kinases may be required for the activation of fos and jun genes, while kinase activity at later time points may be important for activation of the transactivating capacity of the Fos and Jun proteins. Consequently, the TCR signal alone or together with LFA-3 costimulation does not affect JNK-1 and does not cause a synergistic effect in the activation of JNK1, although its synergy is observed for the activation of p38 and ERK-2.

However, despite the fact that LFA-3 costimulation could increase the activity of ERK-2 and p38, only B7-1 costimulation correlates with high levels of IL-2 production, proliferation, transcriptional upregulation of IL-2 and INF-γ promoter activities, and the full induction of all three MAPKs. The high levels of proliferation observed in LFA-3 costimulated T cells imply that alternative pathways are involved in the activation of T cells acting as an optional signal two to activated T cells for clonal expansion and citokine production.

The signal delivered by CD28 pathway could account for a paracrine production of the IL-2 growth factor leading not only to clonal expansion of CD4+ T cells, but also to the activation of other immunological cells such as B cells, CTL and NK cells. Our studies also clearly demonstrate a requirement for B7-1 costimulation for the full induction of transcriptional activity of the IL-2 and IFN-γ. The results suggest specificity of the JNK 1 kinase when B7-1 is applied as the second signal. The strong correlation between JNK-1 activation and transcriptional activity induced via the CD28/B7-1 pathway clearly supports a major role for this pathway in T survival, IL2 production, and proliferation, despite use of alternative pathways such as CD2/LFA-3 and LFA-1/ICAM-1 to deliver signal two.

5. CONCLUSION

It is concluded that the integration signals that lead to T cell activation and cytokine production may occur through the JNK/ERK pathway induced by B7-1 costimulation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 2
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Comparison of PERI-Operative and Post-operative Events: Spinal Anesthesia versus General Anesthesia for Open Cholcystectomy

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ABSTRACT

Objective: To investigate perioperative and postoperative events as well as feasibility, effectiveness, safety of patient and surgeons satisfaction for open cholecystectomy under spinal anesthesia (SA) compared to General Anesthesia (GA).
Material and Methods: All admitted and consented ASA I and ASA II patients of either sex with diagnosed cholelethiasis for elective open chlecystectomy were randomly divided into two groups. During open Cholecystectomy, the SA group received spinal anaesthesia (SA) using 3.00 ml to 3.5 ml of 0.5 percent hyperbaric Bupivacane intrathecally, while the GA group received propofol, Fentanyl Citrate, Atracurium, and Halothane. Other drugs are only used to treat anxiety, pain, nausea and vomiting, respiratory complications, and haemodynamic stability. All open chlolecystectomy performed by right oblique incision. For two days, intraoperative and postoperative events were recorded. The primary areas of research were. Intraoperative complications (hypertension, bradycardia, nausea/vomiting, difficulty breathing, patient and surgeon satisfaction), post-operative painfree interval, PONV, and analgesia requirement
Result: From July 2016 to December 2017, 200 patients with diagnosed cholelithiasis were admitted for open cholecystectomy, with 150 receiving adequate spinal anaesthesia and 50 preferring GA. INTRA-OPERATIVELY, 18 patients in the SA group experienced respiratory difficulty, which was relieved by 100 percent O2 with a ventimask, 39 patients presented with hypotension, which was managed by injection Mephentermine, only 2 patients received injection Ephidrine, 12 patients experienced nausea and vomiting, which was treated with antiemetic (Injection Ondensetron), and 22 patients experienced pain, which was managed by injection tramadol. POST-OPERATIVELY: Both groups of patients were observed for pain free interval and PONV.
Conclusion: Patients undergoing uncomplicated open cholecystectomy under spinal anaesthesia are safer and more effective than G.A. in terms of intraoperative events, post-operative analgesia, PONV, cost effectiveness, and surgeon and patient satisfaction.

Keywords: Perioperative and postoperative events; patient satisfaction; Mephentermine; anxiety.

1. INTRODUCTION

Open cholecystectomy has traditionally been used to treat cholelithisis. Specially in India may be due lake of laparoscopic experience and equipments [1] under Spinal Aanesthesia (SA) [2]. Is an efficient, safe and cost effective alternative to General Anesthesia (GA). In our study, we look at the intra-operative feasibility, effectiveness, and safety of Spinal Aanesthesia, as well as the post-operative pain-free interval, quick recovery, and early mobilisation [3]. GA is generally favored because of its convenience, well studied and understood safety profile. However, general anesthesia can be

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extremely challenging for patients with difficult intubation, obstructive pulmonary and cardiaovascular diseases. GA with its adverse effects on pulmonary functions and associated post operative pain can lead to a prolonged hospital stay and hence affect the cost of hospital stay [4,5,6,7]. Spinal anesthesia is an essential tool in the practice of anesthesia [8]. PONV in conducting open Cholecystectomy [9]. Though GA is a gold standard but it can be extremely cost effective and challenging for patients with difficult intubation, recently low thoracic epidural block [10,11] and combind spinal epidural block [12] have been used frequently in cholecystectomy and found to be safe and effective alternative to GA. With several advantage, infact SA is better choice than GA [13] as patient has lesser effect on respiratory functions, better post operative pain control [14] minimal PONV and lower incidence of deep vein thrombosis [15].

2. MATERIAL AND METHODS

After the institutional ethics and permission of Authority, study was conducted in Malkhan Singh District Hospital, Aligarh, Uttar Pradesh from July 2016 to December 2017, patients admitted with diagnosed Gallstone/Chlolelithiasis for open chlecystectomy of physical status PS I and PS II of either gender between 18 to 60 years of age divided into 2 groups, excluded patients were with acute pancreatitis, cholecytitis, spinal deformity, infection bleeding disorder, and the presence of any condition contra indicating to SA & GA.

After informed consent taken by Nursing Staff/ Anaestheologist from all the patients undergone for open chlolecystectomy, all patients explained properly and standered pre-operative precaution used by given Tablet Diazepam 5mg and Tablet Alprex 0.5mg night before surgery to relieve discomfort and anxiety.

In the pre-operative room 500ml Ringer Lactate Solution (RL Solution) was commenced intravenously and Injection Ranitidine, inj. Perinorm and one dose antibiotics of 3rd generation of Cepherosporine administrated preoperatively to prevent the infection.

After shifting in operative room non-invasive monitoring (Heart rate, Blood pressure, Pulse oximeterly was established and patients were catheterized with Folly's catheter.

Randomized Spinal Anesthesia (SAB) was given with full aseptic precaution in sitting position in L2-L3 space with 26 gauge spinal needle with 3.00, 3.5ml of 0.5% hyperbaric Bupivacaine intrachecally after conforming free flow of cerebrospinalfluid(CSF). The patients was placed in trendelenburg position for 3 to 5 minutes or till the level of sensory block of T4 was achieved, the level of sensory block was assessed with pinprick stimulous. In patients received general anesthesia(GA) injection Atropine or Injection Glycopyrolate+ fentanyl + Midazolam was given in pre-medication, induction was done after preoxygenation for 2-3 mintue with injection Propofol 2 mg/kg. Injection Succinylcholine 1 to 2 mg/kg and OT intubation was done with PVC ETT after checked and fixed ETT, balanced Anaesthesia/ Maintenance Anaesthesia was continued with IPPV+N2O+O2+ Intermitted halothane+ Atracurium. Neuromuscular block was antagonized with Injection Neostigmine 5ml+Injection Atropine Sulphate after the end of surgery.

Open Chlecystectomy was performed by right oblique incision. All patients were monitered haemodynomically and for any complain of pain, vomiting, and respiratory distress throughout the procedure under SA.

Defined hypotension less than 20% of prooperative MAP was treated with injection Mephentermine 6mg I/V and repeated as per need. Heart rate of less than 60 per minute was treated with 0.6mg Atropine, for complain of Hypoxia oxygen(O2) was administered by ventimask at a flow rate of 2 to 4 lt/min and patients were advised to report events such as discomfort, abdominal pain, headache nausea/vomiting. Each events was treated accordingly. There was no case of open cholecystectomy under SA with any specific region converted to GA. Operating surgeon were requested for any technical difficulty associated with procedure during the operation. In post-operative period I/V crystalloid fluid was given for the next 24 hours and all patients were monitored for respiratory distress, heart rate, B.P., Urine output, Pain and PONV. On complain of post-operative pain analgesia

was provided with intramuscular Diclofenac Sodium, post-operative pain was assessed by visual analog scale (VAS=0, No pain) VAS 1-3=Mild Pain, VAS 4-5= Moderate Pain and VAS 6-10= Severe Pain).

If patient could not felt pain relief & persisted for 30 minutes with VAS score more than 3, intravenous Tramadole was given. For severe pain (VAS>6) Injection Butorphanol Tartrate was used intravenously.

The catheter removed and patients were allowed orally for liquid and soft diet the day after surgery. Patients from our hospital usually discharged after removal of stritches on 8th day but on request overall 20-30% patients discharged from the hospital on the 4th day.

The patients discharged within satisfactory condition without any mortality and mobility at the time of discharge.

3. RESULTS

200 patients with diagnosed Cholelithiasi of grade ASA I & ASA II. Out of these 200 patients, 150 patients under went open Cholecystectomy under SAB(SA Group) and 50 patients preferred GA(GA Group) under spinal anaesthesia was performed without any significant difficulty in all patients except only 3 patients in SA group supplemented with Injection Ketamine+Injection Medazolam+Injection Glycopyrolate. As they complaint of dragging sensation during intraabdomenal packing and liver retraction.

3.1 Intra Operative

Hypotension 26%, Abdominal Pain 15%, Breathing difficulty 12%, Breadycardia 14%, Nausea and Vomiting 8% was most frequent incident in SA group which were treated easily by injection Mephentermine 6mg, Injection Tramadol, 100% O2 by ventimask, Injection Atropine, and Injection Ondensetron I/V accordingly 25% patients remain un-eventfull in SA group post-operatively painfree interval was more than 3 Hours in SA group as compared to 1 Hour in GA group.

Table 1.

Sr. No.	Intra-Operative events in SA Group	No. of patients	Percentage of events
01	Hypotension	39	26%
02	Abdominal Pain	22	15%
03	Breathing Difficulty	18	12%
04	Breadycardiya	21	14%
05	Nausea/Vomiting	12	8%
	Total No. of Patients	112	75%
Total No. of Patients in SA group		150	25% uneventful

Table 2. SA Group

Time Interval of pain complain (In min.)	No. of patients with complain of pain	Percentage (%)
180-210 min	42	28.00%
211-240 min	76	50.66%
241-271 min	18	12.00%
271-300 min	14	9.33%
Total Patients:	150	

226.2±26.5 +=31% p<.001

Mean and standard deviation is 226.2±26.5 in SA group and for GA group it is 95.6±25.9 and it is tested by t-test and it is highly significant and $p<0.001$.

It has been tested by chi square test and it is highly significant and p <0.001 Post-operative PONV was 8% in SA group while it was 30% in GA Group. Overall surgeons were satisfied and preferred SA approach for open chlocystectomy.

4. DISCUSSION

Open Cholecystectomy in District hospitals is still preferred and very common under SA compared to laparoscopic Cholecystectomy where expertise and technologies required which are limited[3,18] . In adequate muscle relaxation is one of the most important problem of open cholecystectomy under regional anesthesia causing difficulty in operation [16]. General anesthesia for open cholecystectomy provides adequate muscle relaxation for surgery. Though it may be associated with so many complications as difficulty in intubation traumatize the airway leading to edema and fluid exudation. Introduction of pathogens may leading to respiratory problems. If patient is suffering from co-morbid conditions which may be increase the cost of operation and hospital stay. GA is usually preffered due to adequate muscle relaxation for open cholecystectomy in comparision with spinal anesthesia but an advantage over GA for it can avoid oral and teeth injury, sore throat during laryingoscopy and other hazards[13] and can be used safely in patients with cardiorespiratory co-morbid conditions [17,18].

Table 3. GA group

Time Interval of complaint (In Min)	Pain	No. of patients	Percentage (%)
60-90 min		26	52%
91-120 min		17	34%
121-150 min		04	8.0%
151-180 min		03	6.0%
Total Patients		50	

95.6±25.9 p

Intraoperative hemodynamic changes are common undesired consequences of SA. In our study (26%) patients suffered from intra operative hypotension and (14%) from bradycardia. Occuring hypotension and bradycardia in our patients was easily treated with Inj. Mephentermine and Atropine I/V respectively. In our study no patient has significant pre-existing respiratory disease hence only (12%) patient complained of mild breathing difficulty may be due to surgical manipulation which was easily managed with oxygen supplement. Intraoperatively (15%) patients had abdominal pain while it was (20%) in Laoutid J et al may be due to stretch on mesentery during intraabdominal packing and liver traction, which was managed with gentle retraction of liver and I/V analgesic Inj. Tramadol. In our study the result was compared to Jaouad Laoutid [19] and khan et al. [20] where they also reported longer average Post Operative painfree interval for open cholecystectomy under SA. They managed majority of the patients in SA group by NSAID [2,20]. In our study Inj. Diclofenac sodium was often sufficient to use.

Table 4. PONV in SA & GA group

	PONV	No PONV	Total No. of patients
Spinal (SA)group	12	138	150
GA group	16	34	50
Toatl No. of Patients	28	172	200

$X^2 = 17.9$

PONV was rarely present (8%) in SA group. While it was reported (30%) under GA group. Open cholecystectomy in distric hospital is preferred and very common under SA may be because of feasibility, safety, cost effective, longer post operative painfree interval and minimal post operative nausea and vomiting PONV (8%) in SA group in compared to GA. As it is more costly, post operative painfree interval is short and reported PONV was (30%) in GA group. While Laoutid Jet al PONV was reported 10% in SA group and 50-70% under GA group, especially in laparoscopic cholecystectomy [21,22]. It was the most important that surgical team was very satisfied with the sufficient abdominal relaxation during the operation [2,20].

5. CONCLUSION

In conclusion conducting elective open Cholecystectomy under SA is not only safe and effective but also provide prolonged postoperative analgesia without respiratory problems and PONV.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 3
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Study on "Fleur De Lys" Muscle-Aponeurotic Plication in Abdominoplasty and Lipoabdominoplasty

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ABSTRACT

Muscle-aponeurotic plication is an essential component in abdominoplasty and lipoabdominoplasty because it allows correcting flaccidity and improves abdominal wall deformity. This plication can be vertical, horizontal, or mixed. Vertical plication is the most commonly used form, although there is a growing tendency to use horizontal plication as part of the TULUA technique. We propose the mixed plication in the form of "Fleur de Lys" as a more appropriate form of correction since it corrects the vertical and horizontal flaccidity. The horizontal plication is performed perpendicular to the straight muscles and allows correcting the vertical elongation of the abdominal wall.

Material and Methods: From February 2015 to May 2019, we operated abdominoplasty and lipoabdominoplasty patients associated with the "Fleur de Lys" plication.

Results: In this period, 144 "Fleur de Lys" plications have been performed in 140 women (97.2%) and four men (2.8%), with ages ranging from 25 to 63 years (average 48 years). One hundred thirty-six cases (94.4%) were primary surgeries, and 8 cases (5.56%) were patients with previous abdominoplasty. We had no complications related to the increase in intra-abdominal pressure, only small dehiscences in 5 cases (3.5%) and seroma in 10 cases (7.8%).

Conclusion: The mixed plication technique in "Fleur de Lys" is a more anatomical manner of correcting the muscle- aponeurotic flaccidity because it fixes the vertical and horizontal flaccidity present in operated abdominoplasty and lipoabdominoplasty patients; its execution is technically easy and the results obtained are as expected, with minimal complications.

Keywords: Abdominoplasty; muscle-aponeurotic plication; plication; lipoabdominoplasty.

1. INTRODUCTION

The abdominal wall is the muscle-aponeurotic structure responsible for protecting the visceral content and maintaining the shape of the abdominal contour. Alterations in their tone and integrity, associated with skin flaccidity produce abdominal contour deformity of varying degrees [1,2]. There is a direct relationship between excess abdominal skin and the degree of muscle- aponeurotic deformity; therefore, patients with great skin flaccidity have defects that are more complex in the abdominal muscle-aponeurotic system [1].

The abdominoplasty and lipoabdominoplasty are procedures that allow correcting the skin flaccidity and repairing the muscle-aponeurotic deformities of the abdominal wall; these corrections are carried out by various techniques of muscle-aponeurotic plication, which are classified into three types: vertical, horizontal, and mixed. The musculoaponeurotic layer of the abdominal wall should be

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corrected during abdominoplasty according to the specific deformity that the patient presents with. In this article, the anatomic basis of deformities and defects of the abdominal wall is described. Different degrees of deformities secondary to pregnancy are described as well as congenital muscle malposition [3,4]. The vertical plication is the most used and is performed along the rectus abdominal muscles [1,2,5], several variants have been described, and sometimes, it is associated with plication of the external oblique muscles [5]. On the other hand, the horizontal plication is performed perpendicular to the straight muscles and allows correcting the vertical elongation of the abdominal wall. It was initiated in 1996, with Gerow, et al. [6] proposing aggressive transverse plication at the waist level; later, plication techniques in the infraumbilical region were described such as that described by Villegasin the abdominoplasty with TULUA technique [7].

The mixed plication, unlike the previous ones, allows correcting both the vertical and horizontal elongation of the muscle-aponeurotic system of the abdominal wall, because it combines the techniques of vertical and horizontal plication at the same time. Jackson and Downie initially described it in 1978 [8] who performed periumbilical plication as a "four-legged helix." Later in 1990, Marques, et al. [9] described the "T-shaped plication" with vertical plication associated with a horizontal plication in the upper abdomen designed to correct epigastric prominence. Likewise, in 1999, Abramo, et al. [10] proposed the "horizontal H plication" as a vertical plication associated with a small horizontal plication in epigastrium and a more extensive plication in hypogastrium. Subsequently, in 2004 Cardenas & García [11] proposed the "anchor plication" as a vertical plication in the upper and middle abdomen, associated with a horizontal plication in the lower abdomen, but without intersection of both. In 2005, Serra-Renom, et al. [12] proposed the vertical plication associated with 1 or 2 small "horizontal plications on-demand" in the upper and middle abdomen, and in 2019 Soares published the "crossbow plication" [13] which consists of a vertical and a horizontal plication that intersect at the level of the lower abdomen (Fig. 1).

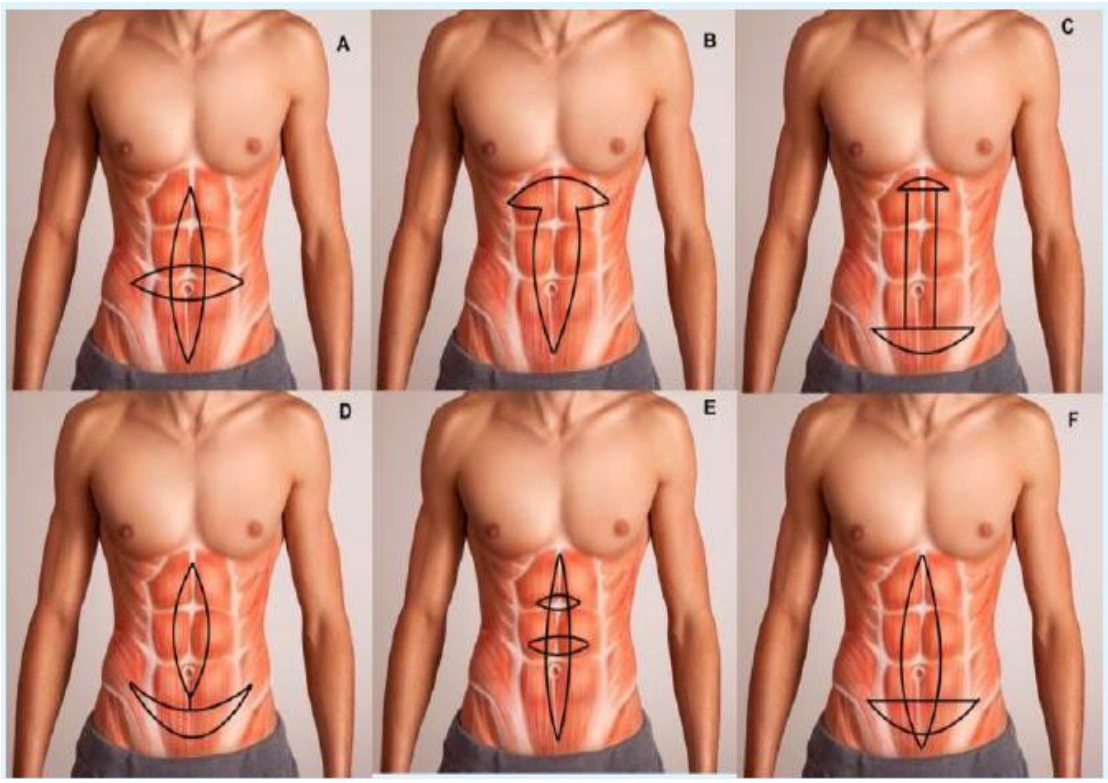

Fig. 1. Mixed Plicatures described. A. Jackson and Downie (1978): Periumbilical plication as a "4-legged helix." B. Marques et al. (1990): Plicature in "T-shape." C. Abramo et al. (1999): Plicature in "horizontal H-shape." D. Cardenas & Garcia (2004): Plicature in "anchor." E. Serra-Renom et al. (2005): vertical plication associated with "horizontal plicatures on demand." F. Soares (2019): Plicature in "crossbow."

We began the mixed plication in 2014. The first cases were in patients who underwent "anchor" abdominoplasty, who due to their great skin flaccidity and aponeurotic muscle required corrections in a vertical and horizontal direction at the same time. In these patients, this type of plication not only helped correct more effectively the great vertical and horizontal flaccidity of their abdominal wall but also contributed to decrease the dead space by approaching the flaps to suture them with less tension. The initial design was in the form of "Tumi", similar to that proposed by Marques, et al. [9], although unlike him, we performed the horizontal plication in the lower abdomen (Fig. 2A); however, this initial design produced a lot of tension at the intersection of both plications in the lower midline, making it challenging to execute. Also, the design of the horizontal plication did not allow correcting the muscle-aponeurotic flaccidity at the level of the iliac fossa. For these reasons, in 2015, the proposal was redesigned, establishing the plication in the form of "Fleur de Lys" for all these cases (Fig. 2B).

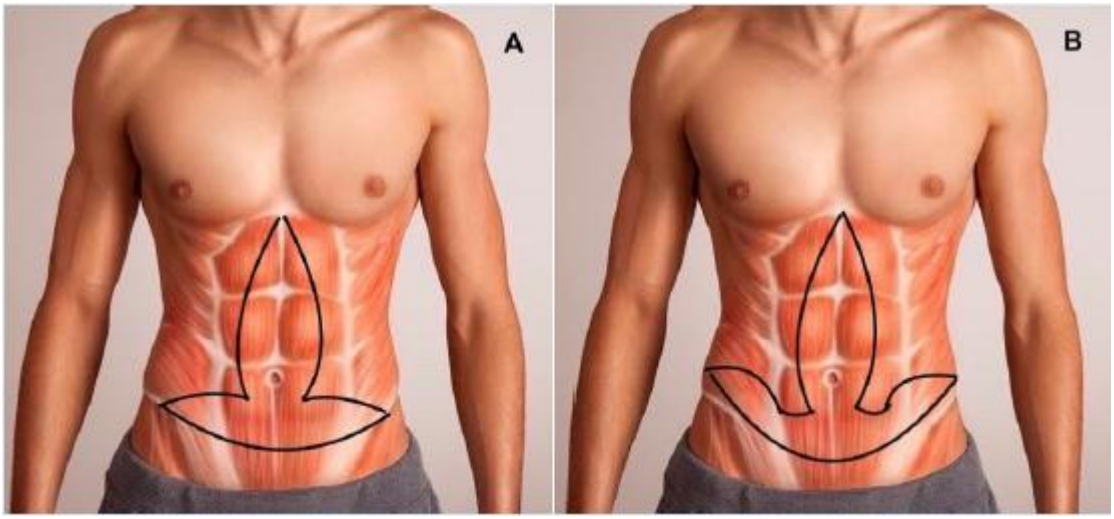

Fig. 2. Mixed plication in "Fleur of Lys." (A) Initial proposal. (B) Current proposal.

Considering that patients with abdominal deformity due to post-pregnancy, obesity, post-bariatric surgery or other causes have muscle-aponeurotic flaccidity of varying degrees, both vertically and horizontally, since 2017 we incorporated this mixed plication in patients who underwent abdominoplasty, lipoabdominoplasty, and lipominiabdominoplasty. This work aims to show the surgical technique and the results obtained using the "Fleur de Lys" muscle-aponeurotic plication in various types of abdominoplasty and lipoabdominoplasty.

2. MATERIALS AND METHODS

From February 2015 to May 2019, operated abdominoplasty and lipoabdominoplasty patients associated with muscle-aponeurotic plication with the technique described were included in the study, excluding cases with the vertical or horizontal plication technique performed individually. The database was reviewed to obtain a list of patients undergoing abdominoplasty or lipoabdominoplasty with the described plications, and then we reviewed their medical and photographic records.

Lipoabdominoplasty patients were operated in a private clinic, and patients with abdominoplasty without liposuction were performed in the Department of Plastic Surgery and Burns at the "Guillermo Almenara Irigoyen" Hospital, both located in Lima, Peru.

2.1 Surgical Technique

Patients underwent operation under general or regional anesthesia. After dissection, elevation, and resection of the abdominal flap, the vertical and horizontal plication is drawn on the exposed abdominal wall, following the proposed design (Fig. 3A). Next, the plication is performed using non-

absorbable Nylon 1/0 sutures, with separate points in the form of "inverted X," such that the knots are buried.

It begins with the horizontal plication with the first point that joins the lower poles of the vertical plication, with the lower midpoint of the horizontal plication at the pubic level (Fig. 3B), continues until completing this horizontal plication (Fig. 3C) after the vertical plication is performed (Fig. 3D). Occasionally, we perform an additional continuous suture, using vicryl 2/0, along the vertical plication to reinforce and avoid palpation of knots. Finally, the closure of the abdominal flaps is performed in two planes, and it ends with umbilicoplasty or neoumbilicoplasty, as appropriate.

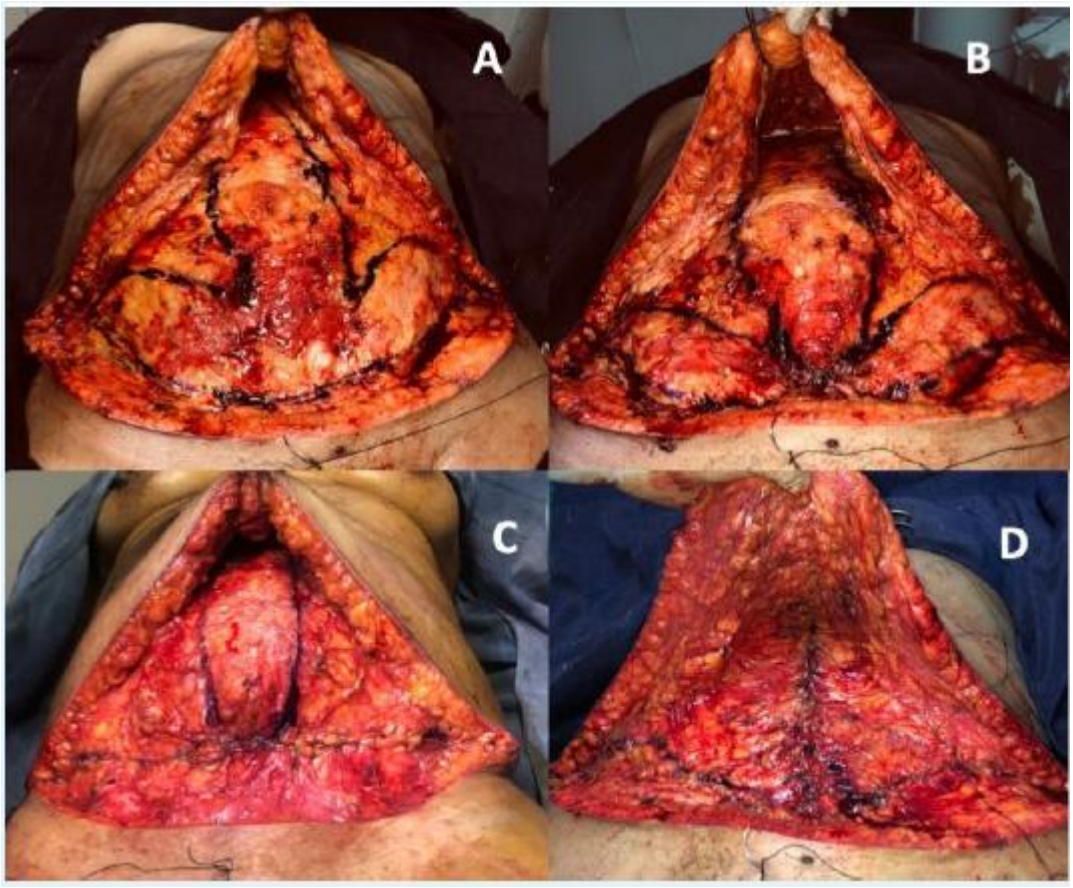

Fig. 3. Surgical technique. A: Design of the plicature in "Fleur de Lys." B. The first point joins the confluence of the vertical and horizontal plication with the midline of the pubis. Note the vertical and horizontal flaccidity to be corrected. C. Result of the horizontal plication, Note the resulting "bulge" that has to be corrected with the vertical plication. D. Final result of the plications, Note the correct correction of the flaccidity abdominal wall

Table 1. Characteristics of the types of body contour surgeries associated with plicatures in "Fleur de Lys"

	Primary	Secondary	Total
Abdominoplasty	7	0	7
Lipoabdominoplasty	76	5	81
Minilipoabdominoplasty	16	1	17
Anchor abdominoplasty	8	0	8
Anchor lipoabdominoplasty	29	2	31
Total	136	8	144

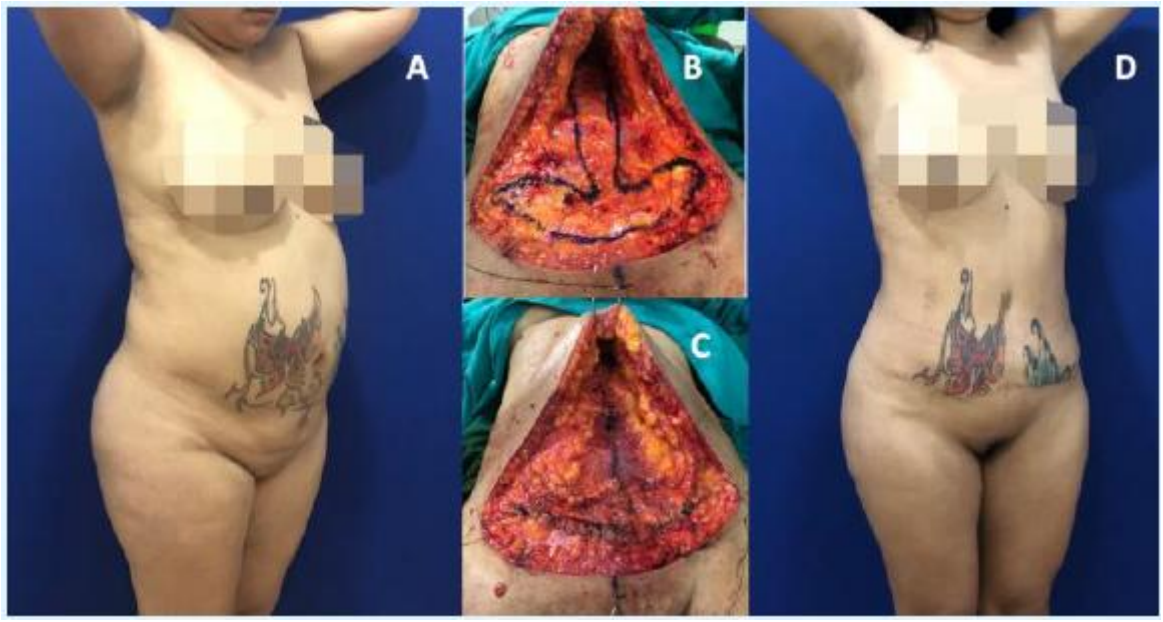

Fig. 4. A 28-year-old female patient, to whom she performed secondary liposculpture, lipoabdominoplasty with anchor plication and neoumbilicoplasty. Preoperative view. B and C. Show the aponeurotic muscle plication performed. D. Results obtained 6 months later

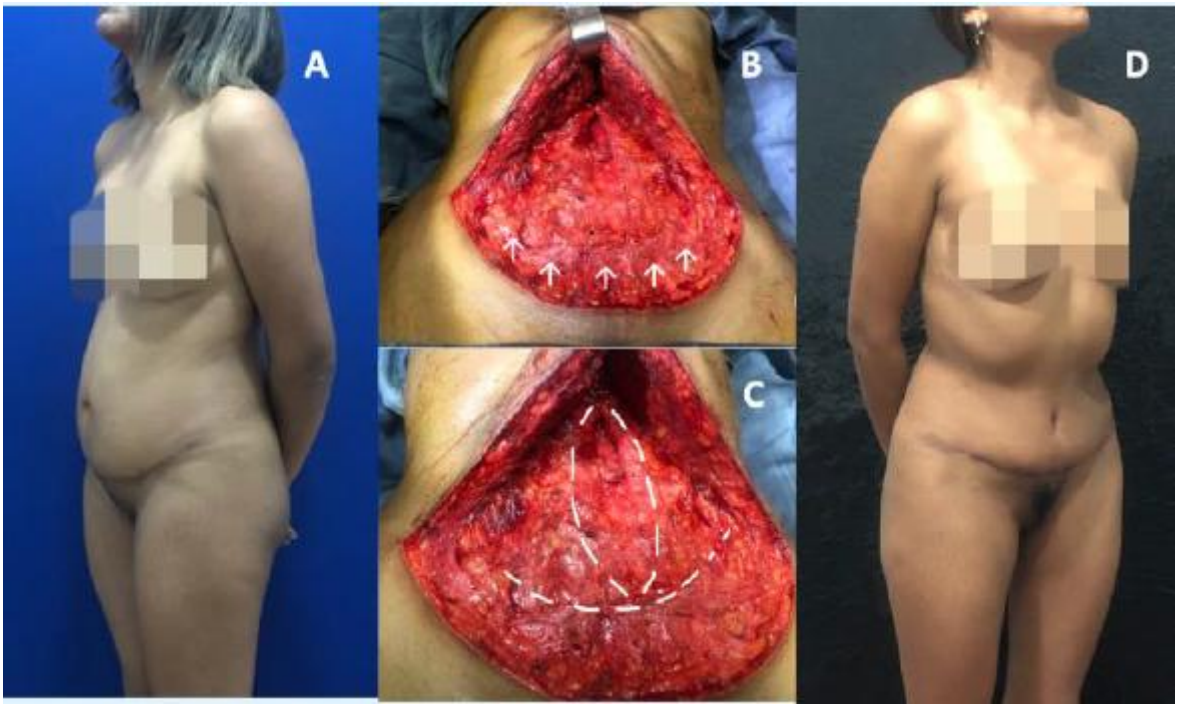

Fig. 5. The patient 32 years operated 6 months before mini-lipoabdominoplasty using the TULUA technique. A. Preoperative photo. B. Horizontal plication performed in the first surgery (arrows). C: Design of the complementary, vertical plicature. D: Photo at 3 months postoperatively

Fig. 6. A 36-year-old patient performed lipominiabdominoplasty with plicature in "Fleur de Lys." Preoperative photo. B. Plication design. C. Plication finished. D. photo of 5 months postoperatively

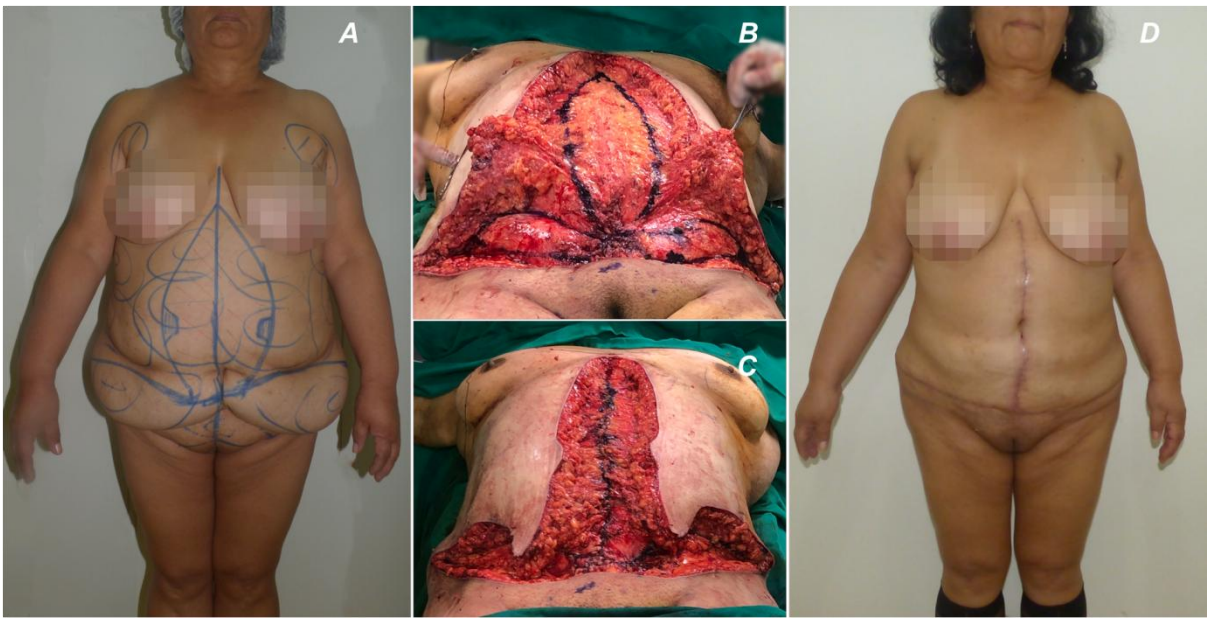

Fig. 7. A 54-year-old patient performed "Fleur de Lys" lipoabdominoplasty with plicature in "Fleur de Lys" Preoperative photo. B. Plication design. C. Plication finished. D. photo of 4 months postoperatively.

3. RESULTS

In the study period, 144 "fleur de Lys" plications have been performed in 140 women (97.2%) and 4 men (2.8%), with ages ranging from 25 to 63 years (average 48 years). 136 cases (94.4%)

were primary surgeries, and 8 cases (5.56%) were secondary abdominoplasty or re- abdominoplasty, of which 5 cases with prior abdominoplasty, 2 cases with prior anchor lipoabdominoplasty, and 1 case of mini-abdominoplasty using previous TULUA technique. 129 plications (89.6%) were performed in the same number of cases of lipoabdominoplasty in its different variants and 15 cases (10.4%) associated with abdominoplasty without liposuction. In all cases, except mini-abdominoplasty, neoumbilicoplasty was performed (127 cases). All 129 cases of lipoabdominoplasty plications were operated at a local clinic, under epidural anesthesia with catheter: 81 plications (62.8%) in patients with lipoabdominoplasty with lower abdominal incision, 17 plications (13.2%) in patients with lipominiabdominoplasty with navel replacement and 31 plications (24%) in patients with anchor lipoabdominoplasty. The 15 cases of abdominoplasty without liposuction were performed in the Department of Plastic Surgery and Burns at the "Guillermo Almenara Irigoyen" Hospital, all of them under general anesthesia, 07 plications associated with abdominoplasty with lower incision and 08 cases associated with anchorabdominoplasty.

There were no complications related to the increase in intra-abdominal pressure. Small dehiscences were described in 5 cases (3.5%) and seroma in 10 cases (7.8%) that were managed conservatively withoutaffecting the final results.

4. DISCUSSION

Patients who request abdominoplasty tend to have excess skin and abdominal wall flaccidity of varying degrees. However, in the beginning, the abdominoplasty was limited only to the management of skin flaccidity without giving importance to the navel or the abdominal wall. In 1967, Callia introduced the medial plication of the straight muscles to improve the abdominal contour in post-pregnancy patients, since then, various techniques and plication variants involving the fascia of the straight muscles [1,2], and in some cases, the external oblique muscles [5] have been proposed.

The proposed technique is a mixed plication in the form of "fleur de Lys" combining horizontal and vertical plication to anatomically correct the flaccidity of the abdominal wall, as this flaccidity is resulting from the elongation of the fibers from the muscle-aponeurotic system both vertically as horizontally. The technique can be used in various types of abdominoplasty and any degree of flaccidity, even in severe cases, avoiding the use of meshes and/or plications of the external oblique muscles. Another advantage offered by this technique is that it allows reducing the dead space (especially in anchor abdominoplasty), and it approximates the resection edges, which allows a closure with less tension. Similarly, the horizontal plication produces pubic lifting as described by Andre FS [14], and its lateral design allows the medial and inferior advance of oblique muscles to allow a better contour in these areas.

In 1978, Jackson and Downie introduced the mixed plication [8] performed at the periumbilical level in the form of a "four-legged helix." Later in 1990, Marques, et al. [9] described a "T-shaped plication"; and years later in 1999, Abramo, et al. [10] proposed the "horizontal H plication", and in 2015, Serra-Renom, et al. [12] proposed the vertical plication with horizontal plications "on- demand". Although these proposals achieved the aim of correcting the muscle-aponeurotic flaccidity, their execution requires an extensive dissection of the abdominal flap, going against the current principles of the abdominoplasty, which is the limited dissection of the abdominal flap that allows preserving the perforating vessels, and decreasing the risk of necrosis and seromas.

Likewise, in 2004, Cardenas & García [11], and 2019, Soares [13] published mixed plication techniques similar to the one proposed in this work, although with some visible differences. Thus, Cardenas & Garcia described an "anchor plication" consisting of a vertical plication in the upper and middle abdomen, associated with a horizontal plication in the lower part, without intersection of both, as two independent plications, unlike our proposal, which is continuous and non-independent. On the other hand, Soares published the "crossbow plication." In this proposal, the vertical plication intersects and exceeds the horizontal plication at the lower abdomen level; this is the difference with our proposal. Also, the location of the horizontal plication is more superior, and the design of the horizontal plication does not efficiently correct flaccidity in that area.

Our priority is to perform the horizontal plication first. At the end of this phase, we observe an abdominal "bulge" (Fig. 3C), which occurs above this plication and is directly proportional to the degree of diastasis of the rectus muscles. We consider that this occurs due to the rearrangement of the visceral content in the new reduced abdominal space, the same that is observed when we perform abdominoplasty with muscular diastasis using only horizontal plication, such as the TULUA technique, as occurred in the case of Fig. 6, which was corrected with a complementary vertical plication becoming a mixed plication like the one we propose in this study.

On the other hand, we must consider that the horizontal plication displaces the position of the navel in a caudal direction; therefore, the possibility of performing neoumbilicoplasty should be considered in the applicable cases. For several years, we have carried out neoumbilicoplasty in all our cases of abdominoplasty and lipoabdominoplasty requiring umbilical transposition. This allows not only to reconstruct a new navel by discarding the original but also to optimize the execution of the vertical plication since it can be performed uniformly without the fear of "hanging" the umbilical stem, as we have shown in the clinical cases presented.

We have not registered any associated cases of breathing problems or other related to increased intra- abdominal pressure. Under normal conditions, the intra- abdominal pressure is subatmospheric, although the muscle-aponeurotic plication is a procedure that increases the intra-abdominal pressure by reducing the volume of the continent. Cases of symptoms of abdominal hypertension, respiratory compromise, and gastroesophageal reflux have been reported in patients with rectus plication. However, few studies have measured intra-abdominal pressure and correlated with symptomatology, with contradictory results. Losken, et al. [14] has shown a significant increase in intra-abdominal pressure (20 mmHg) after the use of the TRAM flap, especially bipediculate, with primary closure of the abdominal wall, which exhibited severe thromboembolism problems. In contrast, Al-Basti, et al. [15] carried out intra-abdominal pressure measurements before and after plication in obese and multiparous patients. They found an increase in intra-abdominal pressure, but without clinical significance; in the same way, through intravesical measurements, Huang, et al. [16,17] concluded that there is an increase in intra- abdominal pressure during the abdominoplasty, but it was not clinically significant. Marin VJA, et al. [18] have shown that the increase of intra-abdominal pressure post- abdominoplasty with plication is less than 10 mmHg. Usually, pressures up to 10 mmHg or 13.6 cm H_2O do not cause any hemodynamic or respiratory changes and pressures greater than 20 mmHg are considered intra- abdominal hypertension.

Seroma is the most common postoperative complication in abdominoplasty and lipoabdominoplasty. For prevention, various surgical alternatives have made developed, although their effectiveness is variable. Seretis, et al. [19] conducted a systematic review and meta-analysis to evaluate the effects of preventive surgical measures on patients with abdominoplasty, finding strong positive evidence in their reduction using preservation of the fascia scarp, tissue adhesives or progressive tension sutures. Other published works reinforce the importance of preserving the fascia of Scarpa in the prevention of seroma [20,21] among others; although Tourani, et al. [22] based on an anatomical study of abdominal lymphatic vessels in fresh cadavers conclude that the preservation of the Scarpa fascia in abdominoplasty would not preserve the lower abdominal lymphatic collectors, and, Har-Shai, et al. [23] they argue that the presence of an "adhesive interface" between the deep adipose compartment and the abdominal flap could explain the contradiction between the clinical success of seroma reduction with the preservation of the scarp fascia and the new find in the abdominal wall lymphatic anatomy. The plicature technique proposed in this work does not allow preserving of the fascia of Scarpa. However, our incidence of seromas (7.8%) is similar to the average of abdominoplasty patients using prevention measures, and who have been published [19]; in fact, these positive results are due to two reasons: on the one hand, the decrease of dead space by the double musculoaponeurotic plication, and, to the adhesion points or "progressive tension sutures" of the abdominal flap to the fascia that we perform in all our cases. The effectiveness of these adhesion points has been demonstrated in various published works [24,25] and systematic and meta-Analysis reviews [19,26].

5. CONCLUSION

The mixed plication technique "Fleur de lys" is a more anatomical manner of correcting the muscle-aponeurotic flaccidity, because it corrects the vertical and horizontal flaccidity present in patients who will undergo abdominoplasty and lipoabdominoplasty; its execution is technically easy and the results obtained are as expected, with minimal complications.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 4
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Dilemma in Breech Delivery: A Review

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ABSTRACT

The incidence of breech presentation seems to have remained steady at about 3- 5 % of singleton pregnancies after 37 weeks of pregnancy. However the rate of caesarean section for breech has risen significantly. Decreasing rate of vaginal breech delivery may mean increased morbidity and mortality for the mother and baby. It may also mean that the expertise of conducting a breech delivery may be dwindling.
Contrasting the findings of The Term Breech Trial which advocated caesarean section for breech births, the newer studies like the PREMODA study, FRABAT study, RCOG, FIGO and others have not shown any increase in risk to the baby or mother after vaginal breech delivery. They have suggested that planned vaginal Breech Birth can be a safe option for the patient to choose, in properly selected patients. Patient education and stressing the importance of regular antenatal clinic check-ups will help take the correct and informed decision of either a planned vaginal delivery or elective caesarean section.
The art of external cephalic version and the art of conducting vaginal breech delivery needs to be relearnt and percolated to the junior staff.

Keywords: Breech delivery; caesarean section; external cephalic version; planned vaginal breech birth.

1. INTRODUCTION

The incidence of breech presentation is about 3- 5 % of singleton pregnancies after 37 weeks of gestation [1]. However the rate of vaginal breech birth has steadily declined in the last several years [2]. There is lot of debate regarding the best way of managing term breech presentation and the mode of delivery.

Diagnosis of breech is made late in pregnancy, because the baby keeps moving in the mother's abdomen till term which is known as changing lie and this is a common occurrence when the gestation is preterm. When breech presentation is diagnosed between 35 and 42 weeks, it creates stress for the pregnant woman because decision about mode of delivery of management needs to be made at this time [3].

Various management options are External Cephalic Version (ECV), caesarean section and vaginal birth. There are various factors affecting the choice of delivery method which include education, economic strength, anxiety, and self-confidence [4].

The main risk to the fetus is difficulty in the passage of the unmolded after-coming head of breech. As compared with a foetus with cephalic presentation, the breech foetus is at increased risk during labour and delivery. There is risk of asphyxia from cord compression and delay in delivery of the after coming head and of traumatic injury during delivery of the shoulders and head. The debate surrounding the optimal mode of delivery for the breech foetus, focuses on the single clinical question regarding the

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magnitude of risk to the foetus during vaginal birth and how it should be balanced against the increased immediate and future risk of caesarean section to the mother.

Vaginal breech birth is still not offered in many settings. This may be because of lack of expertise in conducting skilful vaginal breech delivery. Another drawback of "elective caesarean for all" policy is the negative impact on training, further reducing the number of practitioners with the required skills and experience to deliver breech vaginally effectively and safely. Even in academic medical centres where faculty support for teaching vaginal breech delivery to staff & residents remains high, there may be insufficient volume of vaginal breech deliveries to adequately teach this procedure [5].

The Term Breech Trial had a significant effect on mode of breech delivery. The study suggested that caesarean section was the safest mode of birth for women having breech presentation despite concerns raised regarding the validity of the study [6-8]. In recent times there are concerns surrounding the global rise in caesarean section rates. Also there are criticisms regarding the methodology and the interpretation of the TBT. This had led to a reconsideration of vaginal breech birth by various authorities.

The positive results of the PREMODA study (Presentation ET Mode d"Accouchement: presentation and mode of delivery) study produced different findings. The PREMODA trial which is often referred to as the "antidote to the Term Breech Trial" was conducted in France and Belgium. They used the same criteria and outcome measures as the Term Breech Trial (TBT), except the decision for a caesarean section (CS) or Vaginal Breech Birth (VBB) was the woman's, not randomized. The PREMODA study investigated 3 groups: planned caesarean section, planned VBB, and unplanned CS during a planned VBB. In total there were 8,105 women in the study. Twenty eight percent of planned VBB ended in a caesarean during labour. They had much less crossover in the planned CS arm (<1%) than in the TBT (10%). The PREMODA trial did not find a significant excessive risk associated with planned vaginal birth as compared with planned caesarean for women with a singleton foetus in breech presentation at term. In light of the PREMODA study, some obstetricians have been calling for a return to breech vaginal delivery [9].

The International Federation of Gynecology and Obstetrics (FIGO), Le Collège National des Gynécologues et Obstétriciens Français (CNGOF), the Royal College of Obstetricians and Gynaecologists (RCOG) and the Society of Obstetricians and Gynaecologists of Canada (SOGC) now support the option of vaginal breech birth [10-12].

FRABAT study 2020 - Out of 37 women with a prior caesarean 19 had a successful vaginal delivery. Neonatal and maternal morbidity and mortality were not significantly different in women undergoing successful breech delivery after prior caesarean. The rates of caesarean section were not increased much in women with a prior caesarean section the rate was 49% as compared to the CS rate in primipara 39%. Hence a previous caesarean should not be deter one from planning vaginal breech delivery [13].

Similarly for women who are nearer to their expected date of delivery and in women who become post-dated, elective caesarean section for breech presentations at term is not obligatory [14]. Macrocosmic babies delivering vaginally may be at increased risk of shoulder dystocia and associated complications [15].

2. BREECH CLINIC

The return of focus on vaginal breech birth means that vaginal deliveries need to be well planned. In order to counsel and support eligible women in their choice of mode of delivery and to standardise the care rendered, a 'breech clinic' has been proposed. A 'breech clinic" acts as a dedicated care pathway and as a vaginal breech protocol. A 24-hour on call trained specialist team is advocated, in order to effectively support women and clinicians [16,17].

3. ELIGIBILITY CRITERIA FOR ATTEMPTING A BREECH DELIVERY

a. Highly motivated expectant woman
b. Gestational age more than 37 weeks
c. Estimated fetal weight above 2500 g
d. No hyperextension of the fetal head
e. No cephalo-pelvic disproportion
f. No other indication for caesarean section like two or more previous CS, placenta praevia etc
g. CT scan pelvimetry done and found adequate

4. MANAGEMENT OF LABOUR AND DELIVERY

A detailed history taking and examination is warranted at the time of admission. An ultrasound is performed to confirm the presentation and to exclude hyperextension of the fetal head. If indicated, induction of labour is performed ideally with favourable cervix (Bishop Score > 6–7). Continuous electronic fetal monitoring is must during the labour and if necessary. During the delivery, routine physiological techniques are preferred and no unnecessary maneuvers are done. Giving episiotomy is individualized. Epidural analgesia is advised and used based on maternal request. Currently selection parameters are part of national breech management guidelines. The green-top guideline considers an estimated fetal weight above 3.8 kg a risk factor for vaginal delivery [1,9].

5. NEONATAL OUTCOMES

Breech deliveries are associated with very high perinatal mortality and morbidity. These are due to combination of trauma, birth asphyxia, prematurity, and malformations. The risk of intrapartum fetal death in vaginal breech delivery is 10 fold higher as compared to vaginal cephalic delivery. Overall, the risk of perinatal mortality for planned vaginal breech delivery is approximately 2/1000. Similarly, the risk is 1/1000 for cephalic vaginal delivery and 0.5/1000 for caesarean section after 39 weeks [1].

Some studies have suggested that the increased risk to fetuses that remain in the breech presentation at term may be due to antenatal or other underlying factors that may be associated with the breech presentation. And may not be necessarily due to the mode of delivery [18,19]. In general, breech presentation may have an increased risk of subsequent physical or mental disability in the infant, irrespective of mode of delivery. Also neonates undergoing term breech deliveries may have long term morbidity up to the school age irrespective of mode of delivery [20].

The wide ranges of management policies have been instituted with the aim of reducing perinatal morbidity and mortality. External cephalic version (ECV) is one of such policies. A successful ECV leads to a more favourable presentation and reduces the incidence of breech deliveries, perinatal morbidity, and mortality. This was the reason the Royal College of Obstetricians and Gynaecologists in 2001 recommended that all women with an uncomplicated breech presentation at term be offered an ECV [21-23].

6. CONCLUSION

Decision to deliver the breech baby vaginally is a very tricky decision for both the Doctor as well as the patient. Here counselling of the patient is very important so that an informed decision can be taken. This is especially so in prim gravida women. Decision regarding the mode of delivery will depend on the type of breech, whether it is a frank breech or a complete breech. Also important is age and parity of the woman, gestational age of the fetus, baby size and adequacy of maternal pelvis, presence of previous cesarean section scar etc. breech presentation should be managed at a higher center where proper monitoring of the mother and baby can be done, where facilities of cesarean section are available and also at a place which has expertise in conducting a vaginal breech birth.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Chapter 5
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Study about Cervical Part of Vertebral Artery: An Approach to Its Variant Anatomy and Clinical Significance

Rajani Singh[1*]

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ABSTRACT

Summary: The vertebral artery sprouts from the subclavian artery and is divided into four segments. First three segments are extracranial and located in neck. Hence first three segments of vertebral artery constitute the cervical vertebral artery. The aim of this chapter is to elucidate the anatomical variations in the origin and course of the cervical part of vertebral artery and associated clinical significance. Detailed knowledge of anatomy and its variants is pivotal for the prevention of vascular complications during surgical procedures and while carrying out diagnostic and interventional angiography in the cervical region, while treating cerebro-vascular diseases and of utmost useful for vascular surgeons, radiological and neurosurgeons dealing with neck area.

Keywords: Vertebral artery; variations; vascular surgeons.

1. INTRODUCTION

Vertebral artery originates from first part of subclavian artery. Its course is divided into four parts (Fig. 1);

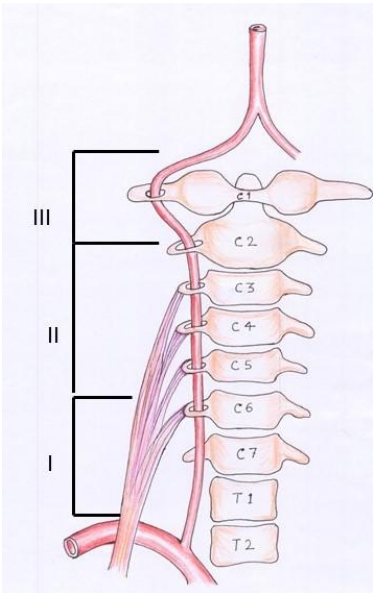

Fig. 1. Showing three parts of cervical vertebral artery

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I: Preforaminal segment, II: Foraminal segment, III: Extradural (atlantic) segment: Preforaminal segment, Foraminal segment, Extradural (atlantic) segment and intracranial segment. Out of these four parts, first three parts are located in neck and fourth part is situated inside the cranial cavity. First three part of vertebral artery forms cervical part of vertebral artery, the normal anatomy of which is

described below: First part also known as Preforaminal segment: After originating it passes superiorly and posteriorly between the longus colli and the anterior scalene muscles. it is related Anteriorly to are the vertebral and internal jugular veins, posteriorly, to the transverse process of C7 vertebra, the sympathetic trunk, and the superior cervical ganglion.

Second part of vertebral artery is also named as Foraminal segment: After passing between the longus colli and anterior scalene muscles, the vertebral artery courses superiorly through the transverse foramina of the C6 to C2 vertebrae. It pursues an almost vertical course up to the transverse process of the axis vertebra. While passing through the transverse foramina, the vertebral artery is related anteriorly to the trunks of the cervical spinal nerves. Also, it is surrounded by the venous plexus, which form the vertebral vein at the lower part of the neck.

Third part of vertebral artery is known as Extradural (atlantic) segment: This segment begins after the artery passes through the transverse foramen of the axis vertebra and it is subdivided into two parts; vertical and horizontal:

Vertical part courses superiorly, crossing the root of the second spinal nerve and then enters the transverse foramen of the atlas vertebra.

After passing through the transverse foramen of atlas, the horizontal part begins. The artery curves medially and posteriorly, passing behind the superior articular process of the atlas and reaches the groove on the upper surface of the posterior arch of the atlas. From there, it passes under the posterior atlantooccipital membrane and enters the vertebral canal. The horizontal part is contained in the suboccipital triangle.

Variant anatomy of different parts of cervical part of vertebral artery: Variations in the origin and course of Preforaminal segment of vertebral artery (Fig. 2).

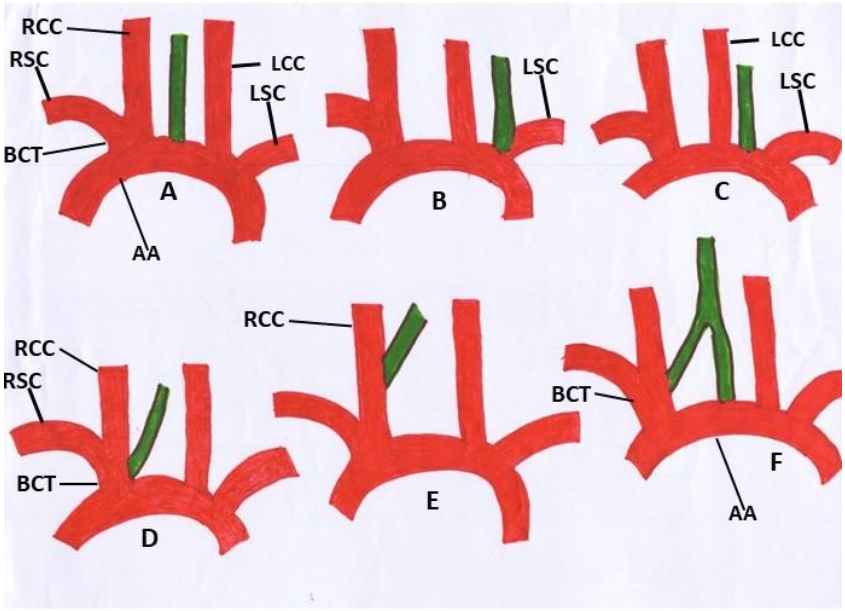

Fig. 2. Showing variant origin of cervical vertebral artery

Cervical vertebral artery is shown in green. A) vertebral artery arising from arch of aorta, B) vertebral artery originating as common trunk with left subclavian artery from arch of aorta, C) vertebral artery budding from arch of aorta between left common carotid artery and left subclavian artery, D) vertebral artery sprouting from brachiocephalic trunk, E) vertebral artery originating from right common carotid artery, F)Duplicate vertebral artery one arising from arch of aorta and other from brachiocephalic trunk and then two vertebral arteries uniting together before entering into foramen transversarium. AA- arch of aorta, BCT- brachiocephalic trunk, RSC- right subclavian artery, RCC- right common carotid artery, LCC- left common carotid artery, LSC- left subclavian artery

Left vertebral artery may bud from arch of aorta between left common carotid and left subclavian artery, between brachiocephalic trunk and left common carotid artery. In addition to this it may arise from arch of aorta as a common trunk with left subclavian artery. Duplicate right vertebral artery sprouting one from Brachiocephalic trunk and another from arch of aorta has also been described in literature. Right vertebral artery from Brachiocephalic trunk and right common carotid artery has also been observed. Left vertebral artery as a common trunk with left subclavian artery is also described in literature [1]. Recent review describes various variations in the vertebral artery [2].

2. VARIATIONS IN ENTRY OF VERTEBRAL ARTERY IN TRANSVERSE FORAMEN AND COURSE OF SECOND PART OF VERTEBRAL ARTERY

Typical entry point of vertebral artery in C6 transverse foramen ranged between 80%- 94.9%.

Anomalous entrance of vertebral artery in foramen transversarium of C7, C5, C4 and C3 ranged between 5.1%-10% with incidences of 10.4%, 7.6%, 2.3% in foramen transversarium of C7, C5, and C4 respectively. Vertebral artery having abnormal origin from arch of aorta usually entered anomalously in foramen transversarium of C5 and C4 vertebra.

Concerning the entrance of vertebral artery in the transverse canal, in 89% the artery followed straight course in this area, in 7.7% it meandered slightly and in 3.1% it formed clear loops [3].

Variations in the course of third part of vertebral artery: Ulm et al. [4] found that half of the vertebral arteries formed a 90° bend at their exit point at the C2 transverse foramen, 37.5% made a slightly more acute angle and 12.5% formed an obtuse bend inside the groove. In the same study, the proximal loop at the level of the third segment were found tethered between the bony C1 and C2 transverse foramina. The proximal loop projected posteriorly in 12 (48%) cases and laterally in 20% cases; in 32% cases there was a small or no loop and the artery coursed in a straight line between C1 and C2 vertebrae. Normally the superior surface of the horizontal segment of vertebral artery lies in close proximity to the lower lateral surface of the occipital bone. Ulm et al. [4] found no separation between the lower surface of the occiput and the superior surface of the third segment of vertebral artery in 12% of cadaveric dissections.

Lang and Kessler [5] studied the course of the atlantoaxial part of the vertebral artery in 65 head and neck halves and presented 4 main types (I–IV) of loop formation in this region. According to their division, the type I which referred to the classical course of the artery was found in 64.6% of specimens. This course was discovered in 64.6% of the cases on the right and in 62.5% on the left. Type II included vertebral arteries forming a loop sideward more or less caudally and slightly dorsally after their exit from the transverse foramen of the axis vertebra was detected in 14% specimens, more on the left (18.8%) than on the right (9.1%). The cases where vertebral arteries presented clear loops in the dorsal direction and then coursed upward to the transverse foramen of the axis vertebra belonged to the type III. This type was observed in 10.8% specimens, more on the left (12.5%) than on the right (9.1%). Finally, type IV was comprised of the arteries which coursed slanting from the transverse foramen of the axis sideward and upward, with more or less clear curves of the vessel in the ventral direction before it approached the transverse foramen of the atlas vertebra. This type occurred in 10.8%; in 15.2% the slanting course was to the right and in 6.3% to the left.

Krayenbühl and Yasargil [3] as well as Schwedt in 1978 described different subdivisions of loop formation of the atlantoaxial part of vertebral artery: slight bendings, small curves, nearly or no loop

formation and moderate looping and so on. Francke et al. [6] subdivided the loops (concave downward and laterally and inside the transverse foramen of C2 vertebra) and pointed out that these bends seem to increase during aging.

As the artery exits the transverse foramen of C1 vertebra, leading towards the suboccipital region, it courses through the groove of the vertebral artery found on the posterior arch of atlas vertebra. an osseous foramen on the posterior arch of the atlas is sometimes present named in the literature as Foramen Arcuale or Ponticulus Posterior [3]. This foramen, which is formed by the ossification of the atlanto-occipital membrane, transforms the groove of vertebral artery in an osseous tunnel inside of which the artery passes through to the suboccipital region.

After entering the suboccipital region, the vertebra; artery gives off spinal and muscular branches, which supply the upper portion of the spinal cord and the suboccipital muscles, respectively.

Clinical significance of Varian anatomy of cervical vertebral artery: Atherosclerosis was found in the prevertebral part of left vertebral artery originating from arch of aorta (Budhiraja et al. [7] but according to Einstein et al. [7] abnormal origin of this artery does not affect the blood flow. However, it may cause difficulties in surgical and endovascular processes involving arch of aorta. The impairment or disengagement of the abnormal vessel might also affect cerebral function. Anomalous vertebral artery may cause interruption of cerebral blood flow during aortic instrumentation, vessel angiography, or surgery on neck and head [8]. Moreover, abnormal budding of vertebral artery from arch of aorta may predispose to cranial aneurysms. In addition to this, there have been reports that anomalies in the branching pattern of the arch of aorta could lead to cerebral anomalies by modifying the pattern of flow in cerebral vessels. Direct origin of left vertebral artery from arch of aorta seems to increase blood flow in left vertebral artery. This direct flow of blood from aorta to brain or absence of equilibrated flow of blood on right and left side at circle of Willis may probably be the cause of greater occurrence of cerebro-vascular diseases in such cases [9].

Although abnormal sprouting of right vertebral artery from Brachiocephalic trunk and right common carotid artery may not produce any symptom still detailed knowledge of the variation is essential during surgical and endovascular intervention to prevent inadvertent injury and confusion [7].

Vertebral artery may occur when vertebral artery enters foramen transversarium of C5 vertebra specifically when it is associated with cervical spondylosis [10]. When placing C2 pars screws or C1-2 transarticular screws the variant anatomy of atlantoaxial segment of vertebral artery should be thoroughly investigated as the procedures may injure this segment of vertebral artery [4]. Moreover, an intraoperative injury to the vertebral artery may result in unpredictable neurological deficits depending on contralateral vertebral artery flow due to excessive bleeding and disruption of cerebral blood flow [11].

The presence of arcuate foramina may compress the vertebral artery as it passes beneath the bony bridge. This compression could result in neurological conditions as vertebrobasilar arterial insufficiency. The hyperextension of the head or manual pressure in this region during cervical manual manipulation may cause vertebral artery compression, especially when arcuate foramen is present, resulting in stenosis [12] and Vanitha [13]. Moreover, the presence of arcuate foramen may also create vertebral artery dissection especially with neck rotation Cushing et al. [14]. Additionally, complaints of neck pain and cervicogenic headache were reported in considerable patients with arcuate foramen Wight et al. [15] Radiographic imaging is recommended for assessing the presence of partial or complete arcuate foramen. In trauma cases, advanced imaging is strongly recommended. In order to minimise the risk of vertebral artery injuries from screw placement, attentive evaluation of dorsal-lateral arch thickness is a prerequisite, since arcuate foramen seems to be a common anomaly.

Tubbs et al. [16] have observed a lateral atlanto-occipital ligament in close association with vertebral artery so in cases of injury of this ligament, may damage the vertebral artery.

Variation in the second and third part of vertebral artery is reported in patients with congenital scoliosis [17].

According to Russo et al. [9], iatrogenic damage to the second segment of the vertebral artery is rare. However, the anomalous course of the vertebral artery may lead to serious complications during anterior cervical decompression surgery with consequential neurological damage or death. Thus, accurate preoperative planning is prerequisite for anterior cervical decompression [18]. Surgical approaches to the posterolateral craniovertebral junction require working in close proximity to the third segment of vertebral artery. Therefore, an injury of this segment may occur during procedures of the craniovertebral junction, as well as while its exposure when treating tumours [3] culminating into serious complications such as vertebral artery occlusion, formation of an arteriovenous fistula, a pseudoaneurysm, or enormous bleeding that could result in stroke or even death. A preoperative three-dimensional computerized tomography (CT) angiography with axial images should be recommended to identify the presence of an anomalous second part of vertebral artery when suspected on magnetic resonance imaging or CT. Delineation of this anomaly may reduce the risk of intraoperative VA injury [19]. Stellate ganglion block and cervical transforaminal epidural block should be done with caution due to atypical courses of the vertebral artery, Nucleoplasty, endoscopic anterior discectomy, and microscopic anterior foraminotomy are performed in areas anterior to the second segment of vertebral artery. For example, in cervical nucleoplasty, an introducer needle is inserted into the affected disc at the anterolateral side of neck after securing routes for the needle by manual transposition of the internal carotid artery, esophagus, and trachea under C-arm guidance. due to atypical courses of the vertebral artery, including retrothyroid courses, surgeons should be aware that the possibility of injury to the vertebral artery increases when a large diameter needle is inserted into the cervical vertebrae [20]. Knowledge of the branching pattern of aortic arch is important during supra-aortic angiography, aortic instrumentation, thoracic and neck surgery [21].

The vertebral artery may be absent or hypoplastic if there is a persistent proatlantal artery. Thus, a proatlantal artery might remain due to abnormalities of vertebral artery development.

3. CONCLUSION

The thorough comprehension and knowledge of the typical vertebral artery and its variations is of great importance for the prevention of vascular complications during minimally invasive diagnosis and treatment of cerebral vascular diseases, when performing both diagnostic and interventional angiography. If the vertebral arteries are not found in their normal position, it can be misinterpreted as the vessels being congenitally absent. This information is important for vascular or cardiothoracic surgical planning. Anomalous origins may lead to altered hemodynamics and predispose the patient to intercranial aneurysm formation. Therefore, in patients with these anomalies, a thorough search for coexisting aneurysms should be undertaken. Thus, thorough and detailed knowledge of anatomical variations of cervical part of vertebral arteries is pivotal to vascular, neurosurgeons and radiologists.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Chapter 6
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Determining the Relationship between Transversus Abdominis Strength and Lumbar Lordosis in Young Adults

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ABSTRACT

Background: The researchers and clinicians are emphasizing on function of Transverse Abdominis (TrA) which is a deepest abdominal muscles because there is an evidence that TrA is recruited independently of the other abdominal muscles in many different tasks and plays an important role in lumbar stability.

Objectives:

1. To assess strength of transverse abdominis muscle in young adults by using pressure biofeedback unit.
2. To measure lumbar lordosis in young adults by using flexi curve ruler.
3. To assess the relationship between the lumbar lordosis and transversus abdominis muscle strength.

Methods: Total 394 subjects were recruited from the constituent institutions of Sumandeep Vidyapeeth with the age range 18-35years. All the subjects, Lumbar lordosis angle and their TrA strength were measured with the Flexicurve and Pressure Biofeedback Unit respectively. Lumbar lordosis angle was calculated using established formula. Average of 03 trials was considered for TrA strength.

Results: Pearson correlation coefficient was -0.18 on correlating Lumbar lordosis angle with TrA strength, -0.09 for age with TrA strength and 0.11 for age with lumbar lordosis angle.

Conclusion: This study concludes that there is negative relationship between lumbar lordosis and TrA i.e. as the lumbar lordosis angle increases, the strength of TrA muscle decreases. Our findings may help health care professionals to better understand the relationships between Transverse Abdominis muscle strength and Lumbar lordosis in young adults.

Keywords: Transversus abdominis; lumbar lordosis; flexiruler; flexicurve; pressure biofeedbackunit; young adults; age; lumbar stability.

1. INTRODUCTION

The upper body is supported by the lumbar spine and it acts as a bridge to transmit the weight from the upper body to the pelvis and lower limbs. Lumbar spine is the first and foremost accountable for posture and stability [1]. Lumbar lordosis (LL) is the basic component of the posture in keeping the sagittal balance [2]. Lumbar lordosis and changes in lumbosacral angle not only affects the lumbar stability but also affects the lumbar muscle strength [3].

Lumbar lordotic curve and pelvic inclination angles in a standing position as per theory should be affected by the lengths of lumbar erector spinae and abdominal muscles. Hence, in a normal standing posture, pelvic inclination angle is related to the lumbar curve, and these angles are related to the strength and length of the abdominal and back muscles [4].

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Recently the researchers and clinicians are emphasizing on function of TrA which is a deepest abdominal muscles because there is evidence that TrA is recruited independently of the other abdominal muscles in many different tasks including upper and lower limb movements as well as ambulation[5]and plays an important role in lumbar stability. The results Himes et al. [5] indicate that both groups were able to contract the TrA with verbal cueing during a side-bridge exercise progression. Because the TrA contracted similarly during exercise in both groups, the association of LBP with the TrA may be because of another mechanism, such as delayed activation in the feed-forward mechanism during activity or a lack of endurance of the TrA.

As the TrA is a digastrics muscle, contraction of it results in reduction of trunk circumference as well as it flattens the abdominal wall in the lumbar region. This leads to increase of intra-abdominal pressure, tensions in the thoracolumbar and anti fascias resulting in spinal stability [6-8]. So, there is significant evidence that TrA muscle plays a crucial role in spinal control [5]. A number of studies have reported the importance of the Transversus abdominis, which contributes significantly to lumbar stability and posture [8-9]. Decreased LM muscle activation, but not TrA muscle activation, is associated with the presence of factors predictive of clinical success with a stabilization exercise program. Our findings provide researchers and clinicians with evidence regarding the construct validity of the prognostic factors examined in this study, as well as the potential clinical importance of the LM muscle as a target for stabilization exercises [10].

Directly palpating TrA is not possible because Internal Oblique is superficial to TrA and limits tactile feedback [7]. So, clinical method to objectively measure the TrA muscle strength is by using pressure biofeedback [11]. A pressure biofeedback unit is a device to objectively assess abdominal muscle function, including TrA activation, during an abdominal drawing-in manoeuvre [7].

Postural evaluation is used to identify spinal alterations or to track the progress of treatment at the various healthcare levels. Flexicurve or flexible ruler is a simple, inexpensive, non- invasive and safe to use devise, which can be moulded according to the contour of the thoracic and lumbar spine curves in the sagittal plane. This is very helpful in clinical and community based setting [12-15].

However, the relationship between Transversus abdominis strength and lumbar lordosis had been studied, but such relationship studies have been insufficiently studied in young adults. Hence, we aim to assess the relationship between TrA muscle strength and lumbar lordosis in young adults.

2. METHODS

2.1 Participants

This is a cross-sectional study with 394 young adults. Subjects with the age group of 18-35 years without any history of low back pain were included in the study. Of these, 325 were Females and 69 were Males. Subjects with any spinal or abdominal congenital deformity, surgery, trauma, infection, neurological pathology and pregnant females were excluded from the study.

2.2 Procedure

The data were collected using convenient sampling technique.

2.3 Lumbar Curvature Assessment Procedure

Subjects were escorted to the procedure room to maintain the privacy of the subject. Subject with barefoot was asked to assume a normal standing posture on a wooden board. His/her lower back was exposed and a dowel was extended mounted on an adjustable stand horizontally until it touches the subject's xiphisternum. This device aids in the control of postural sway while

subsequent measurements were obtained. While the subject is standing, his/her ASIS and PSIS were palpated to confirm the pelvis symmetry and then the spinous processes of L_1 and L_5 was palpated. Then upper border of L_1 and lower border of L_5 were marked with the pen. The subject remained in the normal standing posture and the flexible curve (Fig. 1A) was pressed against the spinous processes of the lumbar spine. The points that intersect the pen marks were recorded. Then the flexible curve was lifted from the spine without changing the configuration of the curve. The convex side of the flexible curve was traced on the plain paper. The points that intersected L_1 and L_5 were marked, and a line was drawn between them. The length of this line (labelled L) was measured using a ruler. Another line (labelled H), representing the height of the curve, was drawn perpendicular from the midpoint of L to the curve and was measured. These two measurements were used to calculate Theta (θ) (Fig. 1B), an index of lordosis, using the following formula:

$$\theta = 4 \times [\text{Arctan } (2H/L)$$

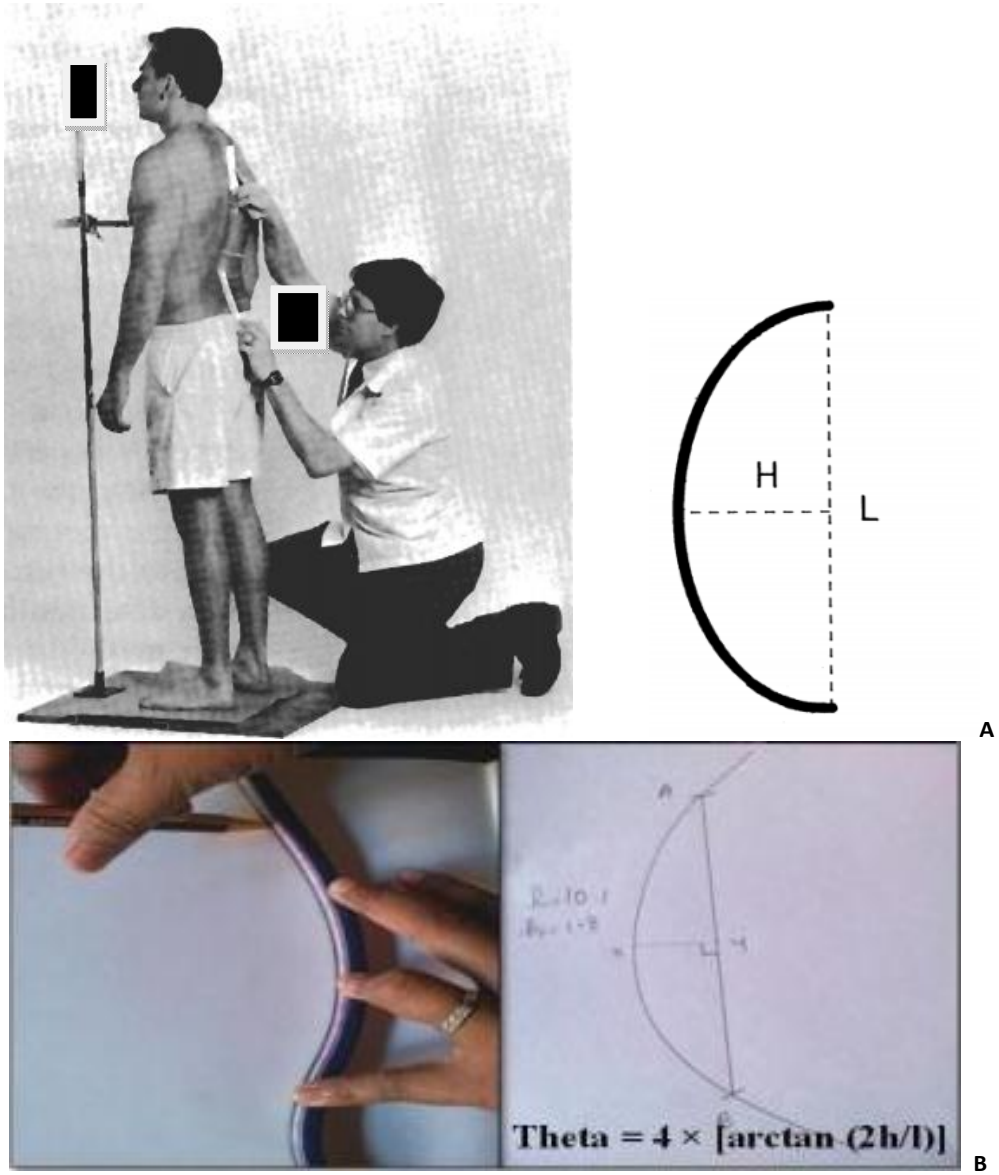

Fig. 1. A: Curve representing the tracing obtained through the use of the flexible ruler; B: The index of lordosis (θ) is obtained by the formula shown where L = the length of the curve and H= the height of the curve and Graphing of the lumbar curve

2.4 Measuring Transverse Abdominis Strength

Patient was positioned in prone lying with arms 90/90, head turned to one side and feet hanging off the end of the table. The pressure biofeedback (Fig. 2) unit was placed horizontally under the abdomen (navel at the centre of the unit) with the lower edge just below the ASIS. The cuff was inflated to 70 mm Hg by the examiner and instructed the patient to perform the drawing-in maneuver while fully relaxing the abdomen and maintaining relaxed breathing without moving the spine or pelvis. If done properly, the pressure dropped by 6 to 10 mm Hg. The therapist had kept a note that the participant can maintain the pressure drop for up to 10 seconds. A 20 sec break was given between each contraction (10sec hold). Muscle endurance (holding or tonic capacity) of the Transversus Abdominis (TrA) was measured by the number of 10 second holds (up to count of 10). Total 03 trials were given to the subjects and the readings were recorded.

2.5 Statistical Analysis

The data were analysed using statistical software and were normally distributed. Descriptive statistics including Mean, Standard Deviation & Standard Error Mean were calculated. ANOVA test was applied to calculate the statistical significant mean difference between the groups with statistical significance kept as 0.005.Post hoc test (Bonferroni correction test) was applied for multiple comparisons between the groups with statistical significance kept as 0.005.

3. RESULTS AND DISCUSSION

3.1 RESULTS

As per the sample size calculations, total 394 participants were included in this study. Mean Age was 20.38 ± 2.54 years. Pearson correlation coefficient was -0.18 on correlating Lumbar lordosis angle with TrA strength, -0.09 for age with TrA strength and 0.11 for age with lumbar lordosis angle.

Fig. 2. Pressure biofeedback

Table 1. Comparison of lumbar lordosis angle and transverse muscle strength

Lumbar lordosis	N	TrA mean	SD	p value
Hypo LL	111	63.22	2.75	**0.0001**
Normal LL	275	60.87	3.13	
Hyper LL	8	62.73	4.82	
Total	394	61.57	3.24	

Table 2. Comparison of Gender with Lumbar Lordosis angle and Transverse abominis muscle strength

Gender	Lumbar lordosis	N	Mean	SD	p value
FEMALE	Hypo LL	101	63.35	2.72	**0.0001**
	Normal LL	221	60.96	3.15	
	Hyper LL	3	66.87	1.40	
	Total	**325**	**61.76**	**3.25**	
MALE	Hypo LL	10	61.83	2.81	0.4530
	Normal LL	54	60.52	3.05	
	Hyper LL	5	60.24	4.36	
	Total	**69**	**60.69**	**3.10**	

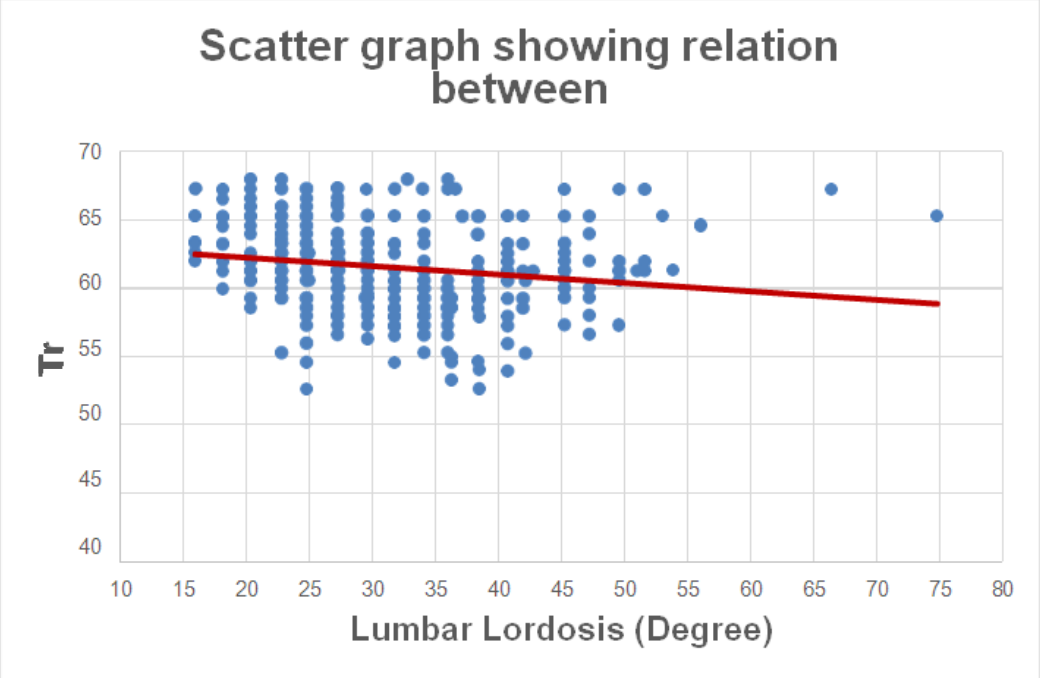

Fig. 3. Scatter graph showing relation between lumbar lordosis and TrA

3.2 Discussion

However all the abdominal muscles play an important role in spinal and pelvis control, Transverse Abdominis muscle has proven its independent role than the other trunk muscles in controlling the spine and its activation is related with the postural demands.[7]This fact is also supported by the study [16] which suggested that Transverse abdominis muscle is to be exercised to increase its muscle mass in order to improve lumbar stability and balance.

To measure the curvature of the lumbar spine and strength of the Transversus abdominis muscle, Flexicurve and Pressure biofeedback unit were used respectively. Nourbakhs et al., [17]

determined from their study that lumbar lordosis angle of females ranged between $42^0 \pm 15^0$ and males' lumbar lordosis angle ranged between $32^0 \pm 10^0$ indicating that females had greater lumbar lordosis angle than males which was in agreement with many other studies [2,18-20]. So, keeping these values as reference values in the study for the males and females lumbar lordosis angle, we segregated the values of the lumbar lordosis (LL) angle into Normal lumbar lordosis angle, Hypo lumbar lordosis angle, and Hyper lumbar lordosis angle. So, according to the lumbar lordosis angle, total 275 subjects had Normal LL, 111 subjects had Hypo LL and 08 had into Hyper LL.

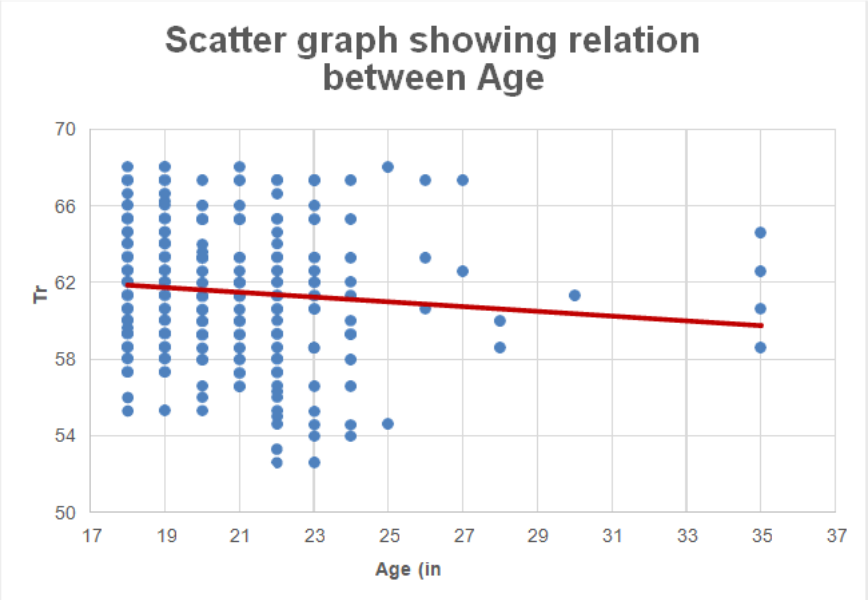

Fig. 4. Scatter graph showing relation between age and TrA

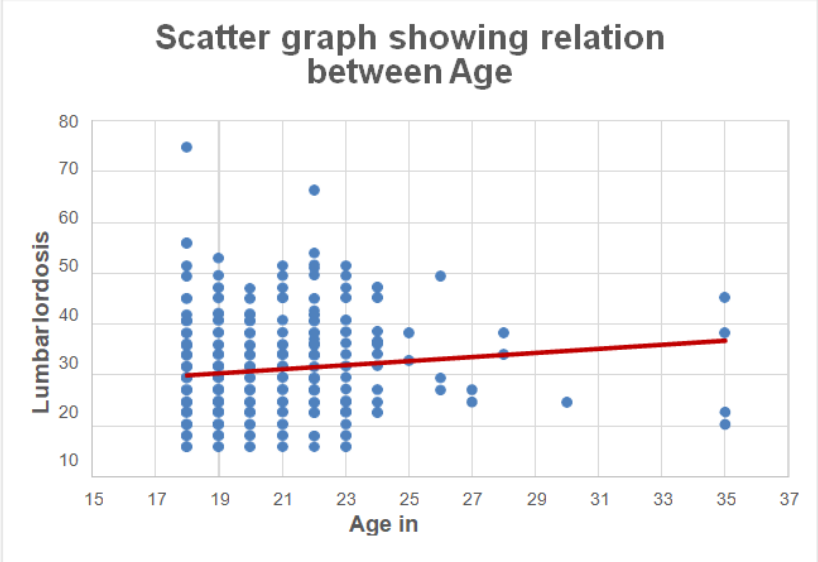

Fig. 5. Scatter graph showing relation between age and lumbar lordosis

Study by Murrie V. et al., [21] indicating that women had greater lumbar lordosis angle reasoning that such variations between the males and females must be genetically studied and it may be due to differences in the pelvis shape and size.

Comparison of lumbar lordosis angle and Transverse abdominis muscle strength, we found that those subjects who were having good Transverse Abdominis (TrA) muscle strength (mean TrA strength 60.87 mmHg) had normal LL angle i.e. females ranged in $42^0 \pm 15^0$ and males ranged in $32^0 \pm 10^0$. While those whose TrA muscle strength was reduced showed decreased or increased lumbar lordosis angle. Our study was in agreement with the study by Jobalia A. et al., [8] who suggested that as the Transverse Abdominis muscle is a tonic, inter-segmental deep muscle, it is expected that its endurance helps in maintaining the spinal stability and hence concluded that Transverse Abdominis muscle strength had a significant role in controlling lumbar stability during postural control. Also according to Kisner et al.,[9] the tonic activity of TrA gives the adequate spinal stability (Table 1).

Comparison of lumbar lordosis angle and Transverse abdominis muscle with the genders, it was noticed that those females who had good TrA muscle strength (mean: 60.96 mmHg, $p<0.0001$) fell in normal lumbar lordosis angle range 27^0-57^0 i.e.$42^0 \pm 15^0$, while those females who had hyper lordosis or hypo lordosis of lumbar spine had reduced TrA muscle strength. This indicated that TrA muscle strength plays an important role in maintaining spinal stability [8]. (Table 2).

In the present study we applied Pearson correlation coefficient (r) to find the correlation of 1. Lumbar lordosis angle with Transverse Abdominis muscle strength, 2. Age with Transverse Abdominis muscle strength, 3. Age with Lumbar lordosis angle. None of the correlation moved independently of each other, they were correlated to each other either positively or negatively.

Lumbar lordosis angle with Transverse Abdominis muscle strength analysed (r) which was -0.18 indicating that there was a weak negative correlation of lumbar lordosis with TrA muscle strength. So, as the lumbar lordosis angle increases, the strength of TrA muscle decreases. Our finding was supported by a study done by Pinto R. et al. [22] which concluded that TrA muscle thickness improves with neutral lumbar postures indicating that muscle strength gets affected when the lumbar spine loses its neutral posture (Fig. 3)

Age with Transverse Abdominis muscle strength indicated weak negative correlation (-0.09). i.e. as the age increases, TrA muscle strength decreases. A study done by Davies P. et al., [22] proved that there is age-related decrease in the performance of the TrA muscle (Fig. 4).

Age with Lumbar lordosis angle, we found the correlation of age with lumbar lordosis as 0.11. It showed positive but weak correlation of age with lumbar lordosis. i.e. as the age increases lumbar lordosis also increases. This study's result was in agreement with study done by Tuzun C. et al., [23] who claimed that lumbar lordosis increases with age (Fig. 5).

So, in this study Pearson correlation coefficient suggested that as the age increases, lumbar lordosis increases and increase in lumbar lordosis results in decrease in TrA performance with the age.

4. CONCLUSION

Deep muscles are particularly important for the lumbar stability. Transverse Abdominis muscle has proven its independent role in controlling the spine. The results of this study have shown negative relationship between Transverse Abdominis muscle strength and Lumbar lordosis in young adults. Our findings may help health care professionals to better understand the relationships between Transverse Abdominis muscle strength and Lumbar lordosis in young adults.

5. LIMITATIONS

1. We targeted small age range, so we were unable to comment on actual changes in lumbar lordosis angle with age, if any.
2. We did not calculate the BMI of the subjects, as BMI plays a significant role in determining the effect of obesity on TrA muscle strength and lumbar lordosis.

3. All the subjects were assessed only once, so data about reliability and responsiveness were not reported in this study.
4.

However, the relatively large sample size and the use of an objective and non-invasive device such as flexicurve and Pressure biofeedback were notable strengths.

6. RECOMMENDATIONS

Reasons for changes in lumbar lordosis angle should be recognized which would be important for clinical application. These reasons will provide platform for future studies.

CONSENT AND ETHICAL APPROVAL

Following ethical approval by the Sumandeep Vidyapeeth Institutional Ethical Committee, subjects who fulfilled the inclusion criteria were recruited. Respondents' written consent has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 7
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Determining Functional End Ranges of Lower Limb Joints in Positions Commonly Used for ADLs in India

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ABSTRACT

Background: In many countries of Asian continent, floor sitting is preferred instead of chair supported sitting. Indian population differs noticeably in its cultural practice and daily tasks which involves squatting and cross-legged sitting on the ground. Squatting is a multiple joint movements performed closed kinetically.
Aim: The purpose of the study was to assess the functional end-ranges of the hip, knee and ankle joints in healthy Indian subjects in positions commonly used for ADLs in India which includes squatting and cross-legged sitting.
Methods: 66 healthy subjects were recruited from rural and urban populations with age range 30- 50 years. Joint ROM of the lower extremities was measured using Universal Goniometer. All the subjects were asked to acquire squat and cross legged positions which were graded.
Results: Our results finding showed that the subjects in cross leg sitting grade 2 (independent CLS) had hip flexion ranges ≥115^0, hip abduction ≥ 41^0, hip external rotation ≥ 42^0, ankle plantar flexion ≥ 46^0, $p<0.005$. For squatting, grade 2 (independent squat) had hip flexion ranges ≥ 113^0,$p>0.005$, Knee flexion ≥120^0, $p>0.005$ and ankle dorsiflexion ≥15^0, $p<0.005$.
Conclusion: From the results, it is suggested that squatting and cross-leg sitting multiple times a day can prevent the early closer of end ranges of the lower limbs. It is important to acknowledge reasons for limitations in ranges, as this is significant for clinical application. By recognizing such reasons one can gain deep understanding to pilot future studies.

Keywords: Floor sitting; ground posture; squatting; cross-leg sitting; lower limb; Asian population; Indian population; range of motion; end ranges.

1. INTRODUCTION

ADLs are remarkably influenced by the culture. In many countries of Asian continent, sitting without external support is still preferred. They prefer floor sitting instead of chair supported sitting. Common floor-sitting static postures acquired by them are squatting, cross-leg sitting and kneeling [1]. They use these postures for eating, personal cleanliness and for religious purposes [2]. Divergent component of motion (DCM) analysis was employed previously based on the linear inverted pendulum flywheel (LIPF) model to regulate the position of the center of mass (CoM) for humanoid robots. In this study, a new extended model is investigated for the DCM analysis by replacing the previous LIPF model, which is tailored for multi-degree-of-freedom (DOF) exoskeletons. This new model is designed to be personalized for each specific user's body by relaxing the assumption of having the total CoM at the hip joint in the previous LIPF model [3]. Accordingly, the exoskeleton has the authority to ensure the postural stability and viability of locomotion in this human-robot interaction (HRI) by adjusting the upper body position using a DCM-based hip correction strategy. Integrating adaptive central pattern generators, the human has enough authority to modify the gait trajectories in real-time, while the amplitude and frequency of walking are constrained to their feasible ranges [4].

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1.1 Squatting

It is floor sitting posture which requires full ankles dorsiflexion, knees and hips flexion [5]. Squatting is a multiple joint movements performed closed kinetically [6]. Squatting is said to be perfectly done when the hips, knees and ankles are in parallel alignment with heels firmly grounded [7].

In India, squatting is most importantly used for toileting activities. In villages, provisions for toilet and bathing activities are inadequate. So, they not only adopt squat position in farm for toileting and for bathing in rivers and lakes [8] but also squatting routine household works, meeting people at home and in religious discourse [8,9]. Whereas in wealthy and urban inhabitants, it is opposite to the above scenario. They squat rarely [10].

Deep squatting capacity is of great significance for the ADLs performed on the ground. It is a structured exercise to strengthen muscles of the lower limb. Deep squat is also a screening test to assess bilateral symmetrical strength and mobility of hip, knees and ankles [5]. Ifa person with decrease in muscle power and/or restricted joint range of motions, performs a deep squat, trick movements and compensations will be there to complete the movement. If hip flexion range of motion is decreased, individual will flex the trunk to gain desired deep squat. Such compensating movements are not suggested as it increases stress on the lumbar spine [5].

1.2 Cross-Legged Sitting

In Asia and Middle East, this is a common position at work, for eating, socializing, leisure or spiritual activities like yoga [9]. Cross-legged sitting is assumed by flexion, abduction and external rotation at hip, flexion at knee and plantar flexion at ankle joint [11]

An individual with restricted joint range of motion still can cross leg sit with compensation. For example, if an individual has reduced knee flexion range of motion, still cross-legged sitting position can be achieved by compensating at hip and ankle i.e. increasing range at hip and ankle joint [1].

In aged, joint ROM is reduced due to aging process resulting in various changes in the joint such as loss of elasticity due to changes in connective tissues, ultimately resulting in restricted squatting and cross-leg sitting [2,12]. In musculoskeletal health system, to evaluate joint motion, most common variable which is measured is Range of Motion (ROM) [12]. To measure the range of motion of lower limb, commonly used device is Universal Goniometer (U.G). It is a double-armed protractor. It is very functional and practical device to measure ROM [2,5,11,12].

Measuring joint ROM precisely is difficult. Even more difficult is measuring ROM during ADLs. So, the literatures so far present are culturally biased. On exploring the existing literature it showed rare studies which focused on squatting and cross-leg sitting positions. Perhaps this partially explains why so few studies have been done that quantitatively investigated ADLs [1].

So, the purpose of our study was to assess the functional end-ranges of the hip, knee and ankle joints in healthy Indian subjects in positions commonly used for ADLs in India which includes squatting and cross-legged sitting.

2. METHODS

2.1 Participants

Total 66 participants were recruited for the study (Fig. 1). The study population had age between 30-50 years including both genders from Dhiraj General Hospital. All the subjects who had any type of neurological, systemic or peripheral pathological disease, history of musculoskeletal injury, any lower limb surgery and pregnancy were excluded.

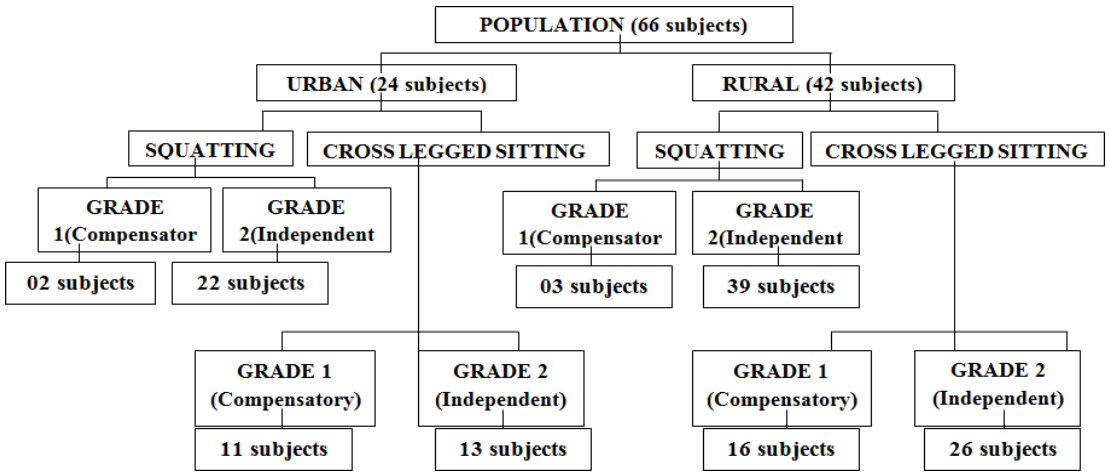

Fig. 1. Flow chart of no. of subjects graded in Squatting and Cross-legged sitting postures

2.2 Procedure

The subject was taken to the procedure room to maintain the privacy. All the functional end ranges of the hips, knees and ankles were taken with the help of universal Goniometer and plastic Goniometer by a single examiner. After the measurements of the end ranges of lower limb, the subjects were asked to perform squatting and cross-leg sitting on the floor and the examiner graded it according to the grading system of squatting and cross-leg sitting.

A. **Squatting** Grades:-

- Grade- 0:- Unable to squat
- Grade- 1:- Abnormal squatting (arm/wall support or heel raise)
- Grade- 2:- Independent full squat

B. **Cross-leg sitting** Grades:-

- Grade- 0:- Unable to cross-leg sit
- Grade- 1:- Deficits in ROM either at hip(flexion, abduction, external rotation) or knee(flexion)
- Grade- 2:- Normal Cross-leg sitting

2.3 Statistical Analysis

All the statistical analysis was performed using SPSS version 22.0 software. Independent T test was used for comparing ROM with squatting grades (i.e. SQ1, SQ2) and cross leg sitting grades (i.e. CLS1, CLS2). ANOVA was used for comparing ROM with BMI of the subjects. In the study, all the statistical tests were performedwith 95% confidence interval.

3. RESULTS AND DISCUSSION

3.1 Results

Total 66 subjects participated in the study, of which 21 were males and 45 were females. Their mean age was 41.24±6.5 years. Of the total 66 subjects, 09 were underweight, 28 had normal weight, 14 were overweight and 15 were obese with the mean BMI of these subjects was 22.35±4.38 kg/m^2.

3.2 Discussion

In India, commonly used positions in day to day living are squatting and cross-legged sitting. Functional end ranges of hip, knee and ankle joints play an important role in deriving these positions.

Table 1. Comparison between ROM and squatting grades

Variable	SQ 1		SQ 2		p value
	Mean	SD	Mean	SD	
HF-R	111.80	3.35	113.61	3.21	0.301
HF-L	112.80	3.70	114.85	3.53	0.289
HE-R	13.80	2.17	13.11	2.27	0.530
HE-L	14.20	4.02	14.90	2.82	0.720
HAB-R	39.80	2.59	39.26	2.97	0.678
HAB-L	39.60	4.04	40.80	2.87	0.547
HADD-R	26.00	1.58	26.54	1.92	0.502
HADD-L	27.60	1.82	28.16	1.60	0.534
HIR-R	41.80	1.10	41.52	1.71	0.627
HIR-L	42.80	2.49	42.75	1.62	0.969
HER-R	39.00	4.18	39.90	3.13	0.660
HER-L	39.60	3.78	40.95	2.88	0.475
KF-R	122.40	6.19	120.44	3.18	0.521
KF-L	121.80	5.54	122.28	3.91	0.858
ADF-R	13.60	1.14	15.82	1.83	**0.008**
ADF-L	15.20	1.64	17.95	1.40	**0.018**
APF-R	43.00	2.45	45.33	2.18	0.100
APF-L	44.80	2.28	47.39	1.67	0.062
AI-R	27.20	1.30	31.89	1.90	**0.001**
AI-L	28.80	2.77	32.97	1.59	**0.027**
AE-R	11.80	1.10	11.98	1.60	0.742
AE-L	10.80	1.64	13.36	1.38	**0.023**

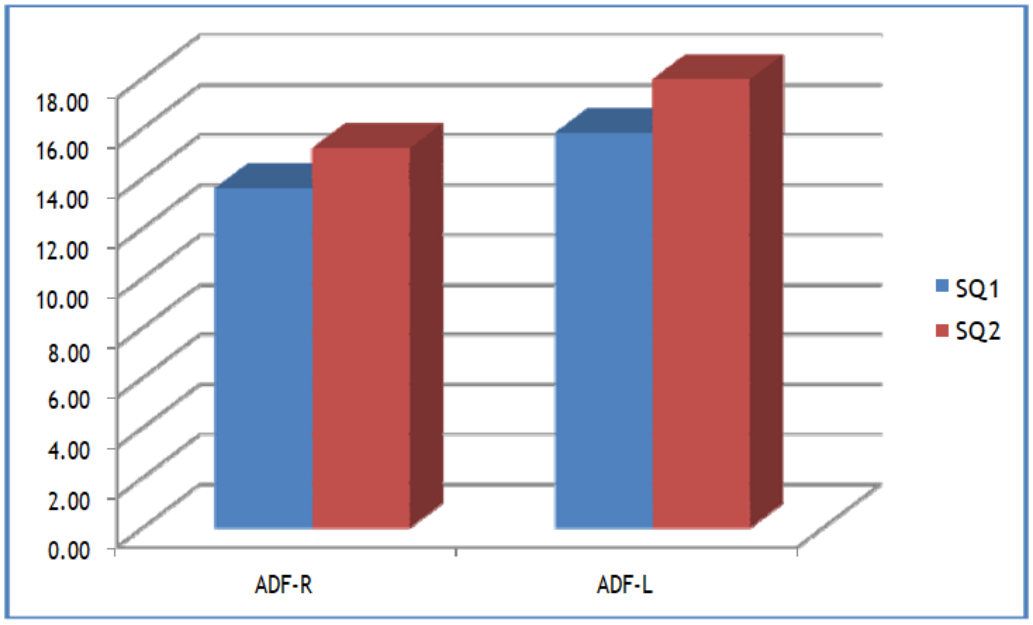

Fig. 2. Comparison of Ankle Dorsiflexion range with Squatting

3.3 Squatting

Squatting is best defined as a posture in which one foot is in complete contact with ground and are extremely flexed, bringing the body down over the feet/foot. This posture demands maximum hip flexion, knee and the ankle joints [13].

Ankle joint: In our study when comparing ROM with squatting grades, all those subjects who were able to squat independently (GRADE 2) had ankle dorsiflexion ≥15° i.e. functional to full ROM. Our findings were in agreement with Bridger RS [14] who stated that if adequate ankle dorsiflexion is available, an individual can bring his/her COG on feet which in result prevents from falling backwards. Regularity of squatting has an additional effect on ankle dorsiflexion rather than squatting for a prolonged time.

Table 2. Comparison between ROM and Cross leg sitting grades

Variable	CLS 1		CLS 2		p value
	Mean	SD	Mean	SD	
HF-R	111.22	2.063	115.03	2.969	**0.001**
HF-L	111.81	2.602	116.69	2.637	**0.001**
HE-R	12.63	1.904	13.54	2.426	0.094
HE-L	13.41	2.531	15.85	2.729	**0.001**
HAB-R	36.70	2.091	41.10	1.875	**0.001**
HAB-L	38.00	1.922	42.59	1.874	**0.001**
HADD-R	25.52	1.528	27.18	1.833	**0.001**
HADD-L	27.56	1.739	28.51	1.412	**0.022**
HIR-R	40.81	1.145	42.05	1.791	**0.001**
HIR-L	42.33	1.593	43.05	1.685	0.084
HER-R	36.81	2.095	41.92	1.869	**0.001**
HER-L	38.00	1.981	42.82	1.554	**0.001**
KF-R	119.74	3.737	121.18	3.170	0.108
KF-L	121.26	4.184	122.92	3.779	0.105
ADF-R	14.48	.893	16.46	1.958	**0.001**
ADF-L	17.11	1.086	18.18	1.730	**0.003**
APF-R	44.30	1.613	45.74	2.479	**0.006**
APF-L	47.26	1.655	47.15	1.967	0.815
AI-R	31.00	1.664	31.90	2.511	0.086
AI-L	33.07	1.730	32.36	2.158	0.141
AE-R	11.74	1.095	12.13	1.809	0.284
AE-L	13.48	1.282	12.95	1.685	0.150

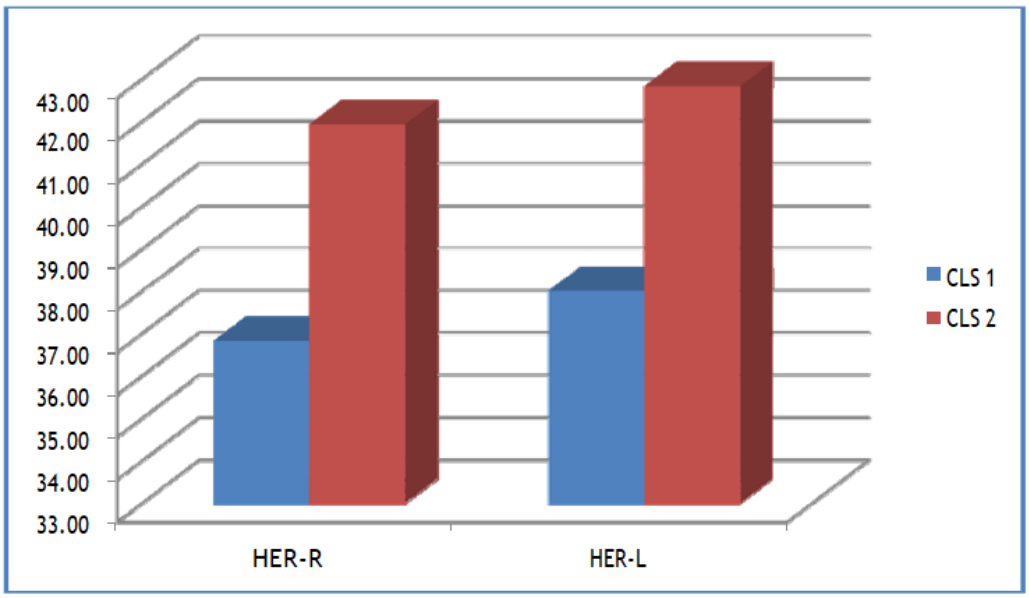

Fig. 3. Comparison of hip external rotation range with cross-leg sitting

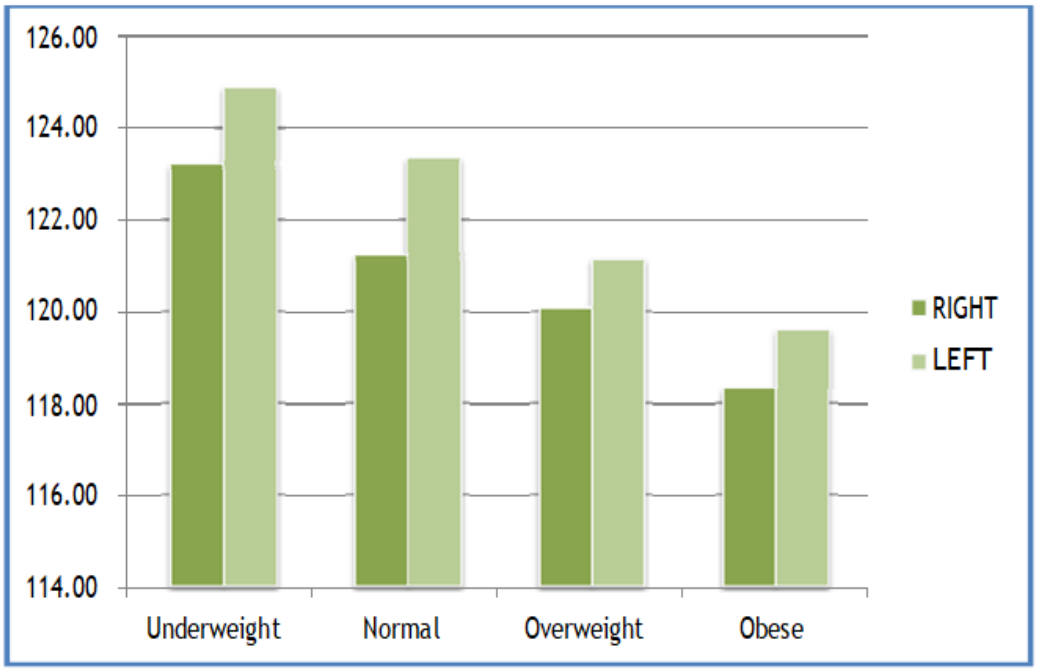

Fig. 4. Comparison of knee flexion range with body weight

Similarly, Butler RJ et al. [15] also emphasized on ankle dorsiflexion range for squatting. He stated that ankle joint is one of the important part of closed kinetic chain during squatting. Restricted ankle joint ranges and inadequate stability of the ankle joint could hinder the performances of the hip and knee joints. We found that when comparing ROM with squatting grades, all those subjects who were able to squat independently (GRADE 2) had ankle dorsiflexion≥15° i.e. functional to full ROM. Also, Kim SH et al., [5] in his study found that restricted ankle dorsiflexion range can limit squatting. Ankle dorsiflexion mobility can alter squatting position. In our study we noticed, that 01 subject had limited dorsiflexion range (12^0) and maintained squat position with heel raise (Fig. 2).

Knee joint: In the literature, knee flexion angles showed consistency and higher ranges, implying that subjects performed deep flexion at knee to achieve squatting position [16]. We also observed consistency and high range in the knee flexion in both squatting grade 1 and grade 2 groups. We found mean knee flexion range 122^0 in our subjects.

Hip joint: In our study, we also found varying hip flexion ROM in both the squatting groups i.e. SQ 1 (grade 1) and SQ 2 (grade 2). The minimum hip flexion range in grade 1 squatting was 111.80°±3.5 and in grade 2 squatting were 113°.16±3.2. These findings were supported by the study of Hemmerich A et al., [16] who concluded that to gain maximum squat,mean hip flexion ROM should be 95.4 ±26.6° (Table 1).

3.4 Cross Leg Sitting

We analyzed that the subjects in cross leg sitting grade 2 (independent CLS) had hip flexion ranges ≥115°, hip abduction ≥ 41°, hip external rotation ≥ 42°, ankle plantar flexion ≥ 46°. This indicates that functional end ranges of hip flexion, hip abduction, hip external rotation, knee flexion, and ankle plantar flexion were maintained (Fig. 3). Our study results were in agreement with Mulholland SJ et al., [1] who reviewed the conclusions of 10 authors throughout the globe on cross-leg sitting and range of motion of lower extremities required for obtaining cross-leg sitting position. Also the values which we found in our study for cross-leg sitting falls within the ranges reported by Kapoor A et al. [11] in his studies (Table 2).

3.5 BMI

Another important analysis we found is of body weight (BMI) and lower limb ROM. In our study, we found statistically significant findings on increase in BMI and decrease in joint ranges. Most affected joint ranges are hip flexion, extension, abduction, hip internal rotation and knee flexion ranges with the increase in the body weight. Our findings were in agreement with Kathiresan G et al., [17], Macedo LG et al., [12] and Roach KE et al.,[18] stated that BMI affects ROM in different ways. So, we suggest that joint ranges decreases as the body weight increases (Fig. 4).

4. CONCLUSION

Functional to near normal end ranges are very important to perform squatting and cross-leg sitting. The results of our study showed significant correlation of lower limb end ranges with squatting and cross-leg sitting positions. BMI influences the range of motion of lower limbs. From, our findings we suggest that squatting and cross-leg sitting multiple times a day can prevent the early closer of end ranges of the lower limbs.

5. LIMITATIONS OF THE STUDY

1. Equal number of both the genders was missed.
2. We also could not recruit equal number of subjects in urban and rural population.
3. We did not group the age to enclose hormonal changes of reproductive and skeletal maturity and perimenopause which are known to influence joint laxity and body mass.

6. RECOMMENDATIONS

1. It is important to acknowledge reasons for limitations in ranges, as this is significant for clinical application. By recognizing such reasons one can gain deep understanding to pilot future studies.
2. The reader must be very observant in generalizing the results as dissimilarities exists between people within countries and its cultures.

ETHICAL APPROVAL AND CONSENT

The study was ethically approved by Institutional Ethical Committee. All the subjects who fulfill the inclusion criteria were included in the study. There informed consent was taken from participants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 8
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Determining the Effect of Polycyclic Aromatic Hydrocarbons Exposure on Cognitive Development in 5 Years Old Children: A Case Study in the Czech Republic

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ABSTRACT

Objectives: To analyze the impact of polycyclic aromatic hydrocarbons (PAHs) in ambient air at the time of delivery and 5 years of age on cognitive development in 5 years old children.
Materials and Methods: Two cohorts of children born in the year 2013 and 2014 from Karvina (Northern Moravia, N=70) and Ceske Budejovice (Southern Bohemia, N=99) were studied at the age of 5 years for their cognitive development related to the exposure to PAHs, determined in the ambient air as the concentration of benzo[a]pyrene (B[a]P) and OH-PAHs metabolites in urine of the newborns at the time of delivery. As psychological tests the Bender Visual Motor Gestalt Test (BG test) and the Raven Colored Progressive Matrices (RCPM test) were used.
Results: Concentrations of B[a]P in the 3rd trimester of mother´s pregnancies were in Karvina 6.1±4.53 ng/m^3, in Ceske Budejovice 1.19±1.28 ng/m^3 ($p<0.001$). Neither the outcome of RCPM test nor BG test differ between children in Karvina vs. Ceske Budejovice, or boys vs. girls. Cognitive development in 5 years old children was affected by the higher exposure to PM2.5 during the third trimester in girls in Karvina.
Conclusions: The rejection rate of part of mothers in our cohorts represents a considerable limitation in our research. We did not observe any significant effect of prenatal PAHs exposure on psychological cognitive tests in 5 years old children.

Keywords: Polycyclic aromatic hydrocarbons; OH-PAH metabolites in urine; psychological tests; cognitive development; bender visual motor gestalt test; raven colored progressive matrices.

ABBREVIATIONS

ADHD:	*Attention Deficit Hyperactivity Disorder*
B[a]P:	*Benzo[a]Pyrene*
BG Test:	*Bender Visual Motor Gestalt Test*
CBCL Method:	*Child Behavior Checklist*
LLE:	*Liquid-Liquid Extraction*
MRI:	*Magnetic Resonance Imaging*
MBD:	*Minimal Brain Dysfunction*
NES2:	*Neurobehavioral Evaluation System*

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OddR: *Ratio of the odds of phenomenon A in the presence of phenomenon B and the odds of A in the absence of B*
PAHs: *Polycyclic Aromatic Hydrocarbons*
c-PAHs: *carcinogenic polycyclic aromatic hydrocarbons*
PM2.5: *Particulate matter < 2.5 μm*
RCPM test: *Raven Colored Progressive Matrices*
SRM: *Standard Reference Material*

1. INTRODUCTION

Already thirty years ago Sram [1] hypothesized that air pollution exposure of the fetus developing in uterus may induce functional changes in nervous system, which may be later expressed as developmental disorders or neurobehavioral impairment.

The first report of the behavioral effects of benzo[a]pyrene (B[a]P) exposure in rats was published by Saunders et al. in 2001 [2].

Polycyclic aromatic hydrocarbons are common carcinogenic and neurotoxic urban air pollutants[28]. The effect of the prenatal exposure to airborne polycyclic aromatic hydrocarbons (PAHs) on neurodevelopment was studied by Frederica Perera in New York for a long term in the Columbia University cohort of non-smoking African-American and Dominican mothers and children. First results indicated DNA damage and impaired fetal growth [3].

This cohort was followed at the age of 3 years by Bayley test [4]. Early cognitive development is vital for an individual's ability to learn, adjust, and take advantage of the opportunities available in various environments[12,27]. Cognitive and psychomotoric development was evaluated at the age of 12, 24 and 36 months. Prenatal exposure of 3.49 ng PAHs/m^3 (less than 1 ng B[a]P/m^3) affected mental development index. Results suggest a possible risk of impairment in language, reading, and mathematics [5]. This cohort was later assessed by Wechsler test at the age of 5 ys. [6], at the age 6-7 years. by CBCL method (Child Behavior Checklist) [7]. Prenatal PAHs exposure affected verbal intelligence quotient score (IQ) and increased symptoms of anxiety, depression and attention problems [7].

Peterson et al. [8] studied the impact of prenatal PAHs exposure on the brain white matter and cognitive and behavioral functions using magnetic resonance imaging (MRI) on 40 children from the Columbia University cohort aged of 7-9 years. They observed the reduced white matter surface of the left hemisphere of children with exposure to PAHs above median 8.2±7.6 ng/m^3, associated with slower information processing speed during intelligence testing, attention problems and increased symptoms Attention Deficit Hyperactivity Disorder (ADHD).

Another prospective cohort was followed in Krakow, Poland [9]. Children were assessed at the age of 5 years by Raven test (Raven Colored Progressive Matrices, RCPM test). Prenatal exposure to PAHs higher than 17.96 ng/m^3 decreased RCPM scores [10]. Transplacental exposures to PAHs were related to shorter head circumference, lower birth weight and lower birth length which may be later related to lower cognitive functions and poorer school performance [11].

These children in Krakow were further tested using Wechsler test. Prenatal as well as postnatal PAHs exposure decreased verbal IQ index. It was the first epidemiological study showing that prenatal PAHs exposure measured as cord-blood PAH-DNA adducts is associated with cognitive dysfunction [12].

Air pollution by PAHs in Krakow is similar to the district of Karvina in the Czech Republic. This was the reason, why we started to study the impact of air pollution on newborns in this district.

In the Czech Republic, the Moravian-Silesian Region is the most polluted region by PM2.5 (particulate matter < 2.5 μm) and c-PAHs (carcinogenic-PAHs), such as B[a]P. These are emitted by heavy

industry and local heating systems. Accordingly, the impact of air pollution on newborns was studied in two districts: the more exposed district of Karvina (Moravian-Silesian Region, Northern Moravia) and the control district of Ceske Budejovice (Southern Bohemia) [13,14]. The study was very complex, analysing the impact of air pollution by PAHs on genetic damage, such as DNA adducts and gene expression, biomarkers of oxidative stress (8-oxodG adducts and lipid peroxidation) and concentration of OH-PAHs in the urine of mothers and newborns. c-PAHs bound to PM2.5 were collected by a High Volume Air Sampler (model ECO-HVS3000, Ecotech, Australia) on Pallflex membrane filters (EMFAB, TX40HI20-WW) for three months during the period of collecting the biological samples [15].

Prenatal exposure to PAHs in cohorts of children from New York (USA) [5-8] and Krakow (Poland) [10,12] indicate the decrease of cognitive functions, intelligence quocient, and decrease of white matter volume in the left hemisphere.

As PAHs concentrations in Krakow (Poland), correspond with PAHs concentrations in Karvina (Czech Republic), we decided to study the impact of PAHs exposure on children from Karvina and Ceske Budejovice (CB) during fetal development at the age of 5 years on their neurobehavioral functions. We tested the hypothesis that high concentrations of PAHs during prenatal development should affect neurobehavioral functions in the children.

2. MATERIALS AND METHODS

2.1 Subjects

The cohorts were created in the summer 2013 and winter 2014 from newborns born in the Ceske Budejovice Hospital, Department of Obstetrics and Gynaecology and Department of Neonatology; and in the Karvina Hospital, Department of Obstetrics and Gynaecology and Department of Neonatology. Newborns were selected from the normal deliveries (38th-41st week) of non-smoking mothers who signed a written consent. Cohorts included 99 newborns (summer) and 100 newborns (winter) in Ceske Budejovice, and 71 newborns (summer) and 74 newborns (winter) in Karvina. The study was approved by the Ethics Committee of both hospitals and the Institute of Experimental Medicine CAS in Prague.

Between November 2018 and November 2019, 199 mothers from Ceske Budejovice district and 143 from Karvina district who provided samples from their children in 2013 and 2014, were approached to take part in psychological testing. Undertaking psychological test was optional. Out of the total amount of 342 potential subjects, 140 refused to take part in the study, and 31 were impossible to contact. In the present study, data from 99 children from Ceske Budejovice and 70 children from Karvina were collected. The final sample therefore included 169 children.

This study was approved by the Faculty of Health and Social Science, University of South Bohemia, Ceske Budejovice.

2.2 Air Sampling and Analysis of Selected Air Pollutants

Particulate matter ≤2.5 μm (PM2.5) was collected by a High Volume (HiVol) 3000 Air Sampler (model ECO-HVS3000, Ecotech, Australia) on Pallflex membrane filters (EMFAB, TX40HI20-WW) in both study locations. The sampling was conducted as previously described [15]. Filters were collected each 3rd day during the urine sampling. Detailed information on air sampling, extraction of organic complex mixtures (EOM) from the filters, and chemical analysis of B[a]P is described in Topinka et al. [15]. Concentrations of air pollutants were expressed in μg/m^3 (PM2.5) and ng/m^3 (B[a]P). Exposure to PM2.5 and B[a]P was calculated for each mother for her last trimester.

When data from HiVol samplers did not cover all trimester, additional published data from CHMI (Czech Hydrometeorologic Institute) were used [16]. The average daily concentrations of PM 2.5μm and benzo[a]pyrene were collected for both localities, Karvina and CB, measure method – beta absorption for PM 2.5μm, gas chromatography with mass detection for B[a]P. Semi-individual

exposure doses were assigned to individual mothers as averages of these values over the period based on the date of delivery.

2.3 Urine samples, OH-PAHs detection

2.3.1 Measurement of urinary creatinine

The creatinine values were used for normalising the urine concentration/dilution in individual samples in order to ensure data comparability. The creatinine concentration was measured using a Jaffe spectrophotometric method according to our previous study [17]. In brief, a coloured complex of creatinine with alkaline picrate was formed and subsequently measured at 505 nm.

2.3.2 Analysis of 11 OH-PAHs in urine

- **Extraction**

  The sample preparation procedure based on liquid-liquid extraction (LLE) with the extraction solvent ethyl acetate and a clean-up step, using dispersive solid-phase extraction (d-SPE) with a sorbent Z-Sep, is described in detail in our previous paper [17].

  Instrumental analysis

  The UHPLC–MS/MS analysis of 11 urinary OH-PAHs was performed, using an Acquity Ultra-Performance LC system, coupled to a triple quadrupole mass spectrometer Xevo TQ-S (both Waters, USA) with electrospray ionisation in a negative ion mode (ESI-). Analytes were separated on a PFP (pentafluorophenyl) Kinetex column, Phenomenex (USA) (100 mm × 2.1 mm × 1.7 µm). Measurement conditions are described in to further detail in our previously published paper [17].

  Quality assurance/quality control and validation

  The validation of the analytical method for analysis of 11 urinary OH-PAHs and the validation of Jaffe spectrophotometric method for the creatinine determination, are described in detail in our previous study [17]. In each set of samples, the method accuracy was checked by using the Standard Reference Material (SRM) 3673 (Organic Contaminants in Non-Smokers´ Urine). Limits of quantification (LOQs) were in the range of 0.01 – 0.025 ng/mL with recoveries ranging between 77-113 % and repeatability 3-16 %.

2.4 Measures of Child Visual-motor Functioning and Intellect

To be able to examine the potential effect of PAHs exposure on cognitive development in 5 years old children, two psychological assessment instruments were used, namely Bender Visual Motor Gestalt test and Raven Colored Progressive Matrices test. From a variety of possible standardized tools these two methods have been chosen having on mind the age of the tested children and the fact that measurement should have been successfully done in one session. Both methods are well received by children and help them adapt to the test situation. 5 years old children from our cohort were tested individually.

In order to assess level of visual-motor functioning in 5 years old children the Bender Visual Motor Gestalt test (BG test) was used. The test focuses on assessing motor functioning, visual perception, and potential developmental or neurological impairments in children and adults [18].

168 children at the age of 5 years completed the test. Each of our 5 years old children was presented with nine cards depicting different geometric shapes. The cards were presented individualy and the tested children were asked to copy the design, trying to make the best reproduction possible. Test results were scored based on the organization and accuracy of the reproduction. This drawing test was well recieved by children and helped them considerably to get used to and feel comfortable with the test situation.

Once the BG test was completed, the children were presented with a non-verbal intelligence test called Raven Colored Progressive Matrices (RCPM test) [19] that was also used in a similar study in Krakow, Poland [10]. The test has been developed and widely used for assessing reasoning and problem solving ability in children between 5 and 11 years, including those suffering some kind of physical or mental impairment. RCPM test consists of three sets of twelve matrix designs with increasing level of difficulty. 167 children at the age of 5 years completed the RCPM test.

2.5 Questionnaire for Mothers

Mothers engaged in the study provided us with information regarding social environment of the family, breastfeeding and eating habits, and child´s medical history. Similarly, the data regarding gestational age, birth weight, birth length, head circumference, and Apgar score were collected in order to be taken into account while analyzing psychological test results.

2.6 Statistical Analysis

There were used two statistical methods for the evaluation of differences in the cohorts. Mann-Whitney U-test (Wilcoxon rank-sum test) was used for direct comparison of RCPM test and BG test results or PAHs related calues between cohorts.

Logistic regression was used for the purpose of estimating the impact of the type of delivery on the scores of RCPM test and BG test as dependent values. Necessary conversion of rough scores of the test values into binary scale, values of OH-PAHs metabolites and EP PAHs values was done by dividing by medians of appropriate group distribution.

The logistic regression quantifies impact intensity to calculated Odds Ratio (OddR), estimates strength of the association between inependent when achieving dependent testing score above median of the group distribution [20].

Calculated OddRs in this analysis show the probability with which children would achieve in RCPM test and BG test scores above the median in their cohorts in association with the PAH exposure from environmental pollution represented either by OH-PAH metabolites in urine or by mean of EP values above median of its distribution, too.

For purpose of exclusion of other possible confounders of estimated impacts, multiple other parameters were tested such as health and social status of mothers, mostly related to maternal questionnaire, like maternal age, maternal ETS (environmental tobacco smoke), various maternal health status parameters, children birth parameters and birth procedures, quantified child illness by categories in period from birth to 2 years. No other statistically significant impact has been found. Impact of the type of delivery and the mother´s education was separately studied [21].

3. RESULTS

Tested confounders are presented in Table 1. Comparing Karvina vs. Ceske Budejovice, in Karvina mothers were younger, ETS exposure was higher during the 1st and 2nd child year, gestation age was longer, birth length was shorter, Apgar 5´ was higher, TBC primovaccination was higher, gastrointestinal diseases in children were more frequent.

Results of the psychological tests in both districts are presented in Table 2. Neither outcome of RCPM test nor BG test differ between children in Karvina vs. Ceske Budejovice, or boys vs. girls.

Concentration of environmental pollution during the third trimester of mother´s pregnancy was calculated from regular pollution measurement according to concentrations determined within 90 days before delivery. Results significantly differed between Karvina and Ceske Budejovice: B[a]P 6.1±4.53 vs. 1.19±1.28 ng/m^3, $P < 0.001$, PM2.5 37.7±14.7 vs. 17.1±4.8 µg/m^3, $P < 0.001$ (Table 3).

All OH-PAH metabolites in urine of children at the time of delivery were significantly higher in Karvina vs. Ceske Budejovice (with exception of 3-OH-B[a]P and 6-OH-chrysene), as they corresponded to a higher PAHs concentrations in ambient air in Karvina (Table 4).When we analyzed the impact of B[a]P as well as PM2.5 exposure in ambient air during the last three months of pregnancy, we did not observe any statisticaly significant effect of the B[a]P exposure on the results obtained by psychological tests, but the exposure to PM2.5 decreased the values of BG test in Karvina in girls (OddR = 0.25, $P < 0.05$) (Table 5).

When we analyzed the relationship between psychological test and PAHs exposure, detected as OH-PAHs in urine at the time of delivery in the period 2013-2014 we did not observe any effects related to the OH-PAHs metabolites values in the time of delivery and the results of RCPM test and BG test in those children aged 5 years (Table 6).

When we analyzed the effect of confounders (Table 1), these confounders did not affect the results of psychological testing. When we analyzed the impact of these confounders on the results of RCPM test and BG test, no effect of these confounders was observed.

61 mothers in our study attained university degree, while 87 higher secondary and 17 lower secondary education. Interestingly, this ratio does not correspond to the values of mapping the level of education in the population according to the results of the census of population conducted in 2011. 36% of mothers in our study achieved university degree compared to the 12% listed by census of Czech population listed [22]. 10 % of mothers willing to take part in their child´s psychological testing attained lower secondary education compared to 17.6% in population according to the census. Mother´s educational level significantly affected the results of psychological tests (Table 7).

4. DISCUSSION

Our results did not support our original hypothesis that high concentration of PAHs in the ambient air in Karvina during prenatal development may affect cognitive functions in the children at 5 years of age. This conclusion is surprising, as the concentration of B[a]P in Karvina during the third trimester of mother´s pregnancies was 6.1 ± 4.53 ng/m^3. When we compared OH-PAHs metabolites in the urine of newborns in the time of delivery, we did not find any effect of any OH-/PAHs metabolites to the cognitive development in 5 years old children. Surprisingly, increased concentrations of PM2.5 during the third trimester affected the results of BG test in girls in Karvina. The rejection rate of part of mothers in our cohorts represents a considerable limitation in our research.

Our results are in discrepancy with the results of studies by Perera et al. [5-7]. B[a]P concentrations in Karvina are at least 5 times higher than in New York. This difference may be partially related to the different ethnicity between African Americans vs. Caucasians as well as social differences between those cohorts in USA and the Czech Republic. As Lovasi et al. [23] already pointed out, child cognitive test scores in the Columbia University cohort were significantly affected by the neighborhood social context. The significant impact of low level of antioxidants as alpha-tocopherol, gamma-tocopherol and carotenoid concentrations at age 6-9 years to neurodevelopment related to PAH prenatal exposure was also observed in the Polish cohort [24]. The quality of diet, vegetables and fruit intake, may be another reason for the discrepancy.

According to various studies, the heritability of intelligence is somewhere between 0.30-0.75 [25]; cognitive abilities can be influenced strongly by the environment, social enrichment, and way of upbringing [26] to name a few. It might be possible that mothers who were more interested in nurturing their children‘s neurodevelopment and cognitive abilities, were thus compensating potential negative effect of the environment by increased care, were also those that were more willing to take part in our study as opossed to the number of those who refused the testing.

Table 1. Overview of tested confounders

		All		CB		Karvina		Boys		Girls	
		N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD
Maternal Characteristics											
Maternal Age	years	168	31.9±4.5	99	32.7±4.4	69	30.8±4.4 *)	78	31.6±4.0	90	32.2±4.9
ETS - pregnancy	cig/day	167	0.06±0.24	99	0.04±0.20	68	0.09±0.29	78	0.06±0.25	89	0.06±0.23
ETS - 1st child year	cig/day	167	0.08±0.27	99	0.03±0.17	68	0.15±0.36 **)	78	0.09±0.29	89	0.07±0.25
ETS - 2nd child year	cig/day	167	0.11±0.32	99	0.06±0.24	68	0.19±0.40 **)	78	0.12±0.32	89	0.11±0.32
Maternal University Education	%	167	0.37±0.48	99	0.34±0.48	68	0.41±0.50	78	0.42±0.50	89	0.33±0.47
Birth Characteristics											
Vaginal Delivery	%	168	0.69±0.46	99	0.70±0.46	69	0.68±0.47	78	0.69±0.46	90	0.69±0.47
Gestation Age	weeks	168	39.8±1.8	99	39.5±1.5	69	40.1±2.0 ***)	78	39.7±1.3	90	39.9±2.1
Birth Weight	G	162	3434±439	97	3464±452	65	3389±417	76	3502±439	86	3374±432 ++)
Birth Length	cm	159	49.7±2.1	94	50.0±1.9	65	49.2±2.3 **)	76	50.1±2.1	83	49.3±2.0 ++)
Birth Head Perimeter	cm	158	34.4±1.4	97	34.4±1.5	61	34.4±1.3	74	34.7±1.5	84	34.2±1.4
Apgar 5'		146	9.9±0.5	88	9.8±0.6	58	10.0±0.1 ***)	66	9.8±0.6	80	9.9±0.3
Other Delivery Complication	%	168	0.05±0.23	99	0.05±0.22	69	0.06±0.24	78	0.09±0.29	90	0.02±0.15
Hyperbilirubinia	%	168	0.09±0.29	99	0.06±0.24	69	0.13±0.34	78	0.08±0.27	90	0.10±0.30
TBC Primovaccination	%	168	0.08±0.27	99	0.04±0.20	69	0.13±0.34 *)	78	0.05±0.22	90	0.10±0.30
Children's Diseases											
GIS	count	168	0.32±0.66	99	0.22±0.56	69	0.45±0.76 **)	78	0.35±0.75	90	0.29±0.57
Viral Diseases	count	168	0.18±0.43	99	0.22±0.46	69	0.13±0.38	78	0.22±0.47	90	0.16±0.39
Otitis	count	168	0.03±0.20	99	0.03±0.17	69	0.03±0.24	78	0.04±0.25	90	0.02±0.15
HCD	count	168	0.29±0.57	99	0.23±0.53	69	0.36±0.62	78	0.28±0.53	90	0.29±0.60
Bronchitis	count	168	2.47±2.58	99	2.25±2.42	69	2.78±2.79	78	2.51±2.68	90	2.43±2.51

*Results of Mann Whitney U-test compare by region *) p ~ 0.05, **) p ~ 0.01, ***) p ~ 0.001 and by gender +) p ~ 0.05, ++) p ~ 0.01, +++) p ~ 0.001*

Table 2. Results of psychological tests

	All		CB		Karvina		Boys		Girls	
	N		N		N		N		N	
RCPM test	168	18.7±4.6	99	18.9±4.2	69	18.3±5.0	78	18.7±4.7	89	18.6±4.6
BG test	169	32.5±15.1	99	32.8±14.5	70	32.1±16.0	79	31.8±16.3	89	33.1±14.0

Results of Mann Whitney U-test, compared by groups $^{*)}$ p ~ 0.05, $^{**)}$ p ~ 0.01, $^{***)}$ p ~ 0.001

Table 3. Concentration of Enviromental Polution in 3rd trimestr of mother's pregnancies for delivery in years 2013-2014

	All		CB		Karvina		Boys		Girls	
	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD
PM 2.5 µm (µg/m^3)	**168**	**24.4±13.3**	**99**	**17.1±4.8**	**69**	**37.7±14.7**$^{***)}$	**78**	**23.8±13.2**	**90**	**24.8±13.5**
B[a]P (ng/m^3)	168	3.18±3.88	99	1.19±1.28	69	6.1±4.53$^{***)}$	78	3.08±3.95	90	3.28±3.85

Results of Mann Whitney U-test, compared by region $^{*)}$ p ~ 0.05, $^{**)}$ p ~ 0.01, $^{***)}$ p ~ 0.001 and by gender $^{+)}$ p ~ 0.05, $^{++)}$ p ~ 0.01, $^{+++)}$ p ~ 0.001

Table 4. Concentration of OH-PAHs in urine (µg/g creatinine) at the time of delivery in years 2013-2014

	All		CB		Karvina		Boys		Girls	
	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD
1-OH-Naphthol	142	0.34±0.47	87	0.11±0.18	55	0.70±0.56$^{***)}$	71	0.33±0.41	71	0.34±0.53
2-OH-Naphthol	142	3.89±3.52	87	2.95±2.38	55	5.37±4.44$^{***)}$	71	3.69±3.29	71	4.09±3.75
2-OH-Fluoranthene	142	0.17±0.15	87	0.11±0.10	55	0.26±0.16$^{***)}$	71	0.14±0.14	71	0.19±0.15$^{++)}$
1-OH-Phenantrene	142	0.42±0.55	87	0.16±0.16	55	0.84±0.67$^{***)}$	71	0.48±0.66	71	0.37±0.40
2-OH-Phenantrene	142	0.23±0.23	87	0.11±0.11	55	0.42±0.23$^{***)}$	71	0.20±0.21	71	0.27±0.24$^{++)}$
3-OH-Phenantrene	142	0.04±0.05	87	0.02±0.02	55	0.08±0.06$^{***)}$	71	0.04±0.05	71	0.04±0.05
4-OH-Phenantrene	142	0.10±0.32	87	0.06±0.13	55	0.17±0.48$^{***)}$	71	0.06±0.14	71	0.14±0.43
9-OH-Phenantrene	142	0.90±1.66	87	0.36±0.69	55	1.74±2.29$^{***)}$	71	0.69±1.40	71	1.10±1.87
1-OH-Pyrene	142	0.07±0.09	87	0.03±0.04	55	0.13±0.11$^{***)}$	71	0.06±0.08	71	0.08±0.10
6-OH-Chrysene	142	0.01±0.00	87	0.01±0.00	55	0.01±0.00	71	0.01±0.00	71	0.01±0.00
3-OH-B[a]P	142	0.45±0.00	87	0.45±0.00	55	0.45±0.00	71	0.45±0.00	71	0.45±0.00
Sum OH-PAH	142	6.14±5.03	87	3.88±2.90	55	9.71±5.60$^{***)}$	71	5.67±4.74	71	6.60±5.29

Results of Mann Whitney U-test, compared by region $^{*)}$ p ~ 0.05, $^{**)}$ p ~ 0.01, $^{***)}$ p ~ 0.001 and by gender $^{+)}$ p ~ 0.05, $^{++)}$ p ~ 0.01, $^{+++)}$ p ~ 0.001

Table 5. Estimated impact of Environmental Polution in 3rd trimestr of mother's pregnancies to psychological testing values

	All			Boys			Girls		
	All	CB	Karvina	All	CB	Karvina	All	CB	Karvina

		OddR (95% CI)	OddR (95% CI)	OddR (95% CI)	OddR (95% CI)	OddR (95% CI)	OddR (95% CI)	OddR (95% CI)	OddR (95% CI)	OddR (95% CI)
RCPM test	PM 2.5µm, 3rd trim	0.62 (0.34-0.14)	0.96 (0.45-2.09)	0.76 (0.28-1.92)	0.63 (0.26-1.52)	1.09 (0.35-3.43)	0.90 (0.24-3.41)	0.59 (0.25-1.39)	0.88 (0.31-2.50)	0.64 (0.17-2.38)
	B[a]P, 3rd trim	0.62 (0.34-0.14)	0.71 (0.32-1.53)	0.67 (0.26-1.71)	0.77 (0.32-1.85)	0.92 (0.29-2.88)	0.90 (0.24-3.41)	0.49 (0.21-1.16)	0.57 (0.20-1.64)	0.51 (0.14-1.92)
BG test	PM 2.5µm, 3rd trim	0.63 (0.35-1.15)	0.52 (0.24-1.13)	0.42 (0.16-1.10)	0.90 (0.38-2.15)	0.78 (0.25-2.44)	0.71 (0.19-2.69)	0.44 (0.19-1.03)	0.37 (0.12-1.10)	0.25 (0.06-0.99)*)
	B[a]P, 3rd trim	0.63 (0.35-1.15)	0.72 (0.33-1.57)	0.48 (0.19-1.23)	0.90 (0.38-2.15)	0.92 (0.29-2.88)	0.71 (0.19-2.69)	0.44 (0.19-1.03)	0.60 (0.20-1.75)	0.32 (0.08-1.24)

*Logistic Regression results *) p ~ 0.05, **) p ~ 0.01, ***) p ~ 0.001*

Table 6. Impact of the 2013-2014 PAH-OH to psychological testing values

		All			Boys			Girls		
		All	CB	Karvina	All	CB	Karvina	All	CB	Karvina
		OddR (95% CI)	OddR (95% CI)	OddR (95% CI)	OddR (95% CI)	OddR (95% CI)	OddR (95% CI)	OddR (95% CI)	OddR (95% CI)	OddR (95% CI)
RCPM test	Sum PAH-OH	1.26 (0.68-2.34)	0.97 (0.44-2.11)	0.87 (0.34-2.25)	1.62 (0.66-3.96)	1.54 (0.47-5.02)	0.46 (0.12-1.83)	0.97 (0.41-2.30)	0.66 (0.23-1.89)	1.78 (0.45-6.97)
	2-OH-Naphthol	1.04 (0.56-1.92)	1.05 (0.48-2.29)	1.10 (0.43-2.86)	1.21 (0.50-2.94)	1.07 (0.33-3.47)	0.75 (0.19-2.92)	0.87 (0.37-2.04)	1.02 (0.35-2.91)	1.40 (0.35-5.54)
BG test	Sum PAH-OH	1.80 (0.97-3.31)	0.86 (0.39-1.89)	0.96 (0.37-2.47)	1.80 (0.74-4.36)	0.56 (0.23-2.43)	0.56 (0.14-2.21)	1.73 (0.74-4.07)	0.91 (0.31-2.64)	1.78 (0.45-6.97)
	2-OH-Naphthol	1.34 (0.73-2.46)	0.80 (0.37-1.76)	1.21 (0.47-3.14)	1.09 (0.45-2.62)	0.75 (0.23-2.43)	0.56 (0.14-2.21)	1.56 (0.67-3.65)	1.70 (0.28-2.34)	2.31 (0.57-9.40)

*Logistic Regression results *) p ~ 0.05, **) p ~ 0.01, ***) p ~ 0.001*

Table 7. Results of psychological tests and the mother's education level

Mother's education level										
	All		**CB**		**Karvina**		**Boys**		**Girls**	
Lower secondary	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**
RCPM test	16	15.0±2.7 ***)	5	15.6±2.9 **)	11	14.7±2.8 ***)	9	15.0±3.4 ***)	7	15.0±1.8 ***)
BG test	17	24.1±12.9 **)	5	25.0±15.7	12	23.8±12.2 **)	10	23.7±15.0 *)	7	24.7±10.2
Higer secondary	N		N		N		N		N	

Mother's education level										
	All		**CB**		**Karvina**		**Boys**		**Girls**	
Lower secondary	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**
RCPM test	51	18.1±4.4 ***)	30	18.4±4.3 *)	21	17.4±4.7 **)	24	18.0±5.0 *)	27	18.1±4.1 *)
BG test	51	31.8±14.3	30	32.4±14.3	21	30.5±14.6	24	29.9±17.4	27	32.9±12.1
University	N		N		N		N		N	
RCPM test	51	20.6±4.5	30	20.4±3.9	21	20.7±5.2	24	20.5±4.0	27	20.6±5.1
BG test	51	35.2±16.0	30	34.5±14.7	21	36.1±17.6	24	35.1±14.5	27	35.3±17.8

*Results of Mann Whitney U-test compare by type of mother's education related to university level degree *) p ~ 0.05, **) p ~ 0.01, ***) p ~ 0.001*

Edwards et al. [10] observed the effect of prenatal exposure of airborne PAHs on 5 years old children in Krakow, Poland, were also using RCPM test, with exposure to sum of PAHs was 17.96 ng/m^3. This exposure was higher than exposure in Karvina.

According to our previous study [21], the effect of the type of delivery, cesarean vs. vaginal, seems to be more significant in affecting cognitive functions in children than prenatal exposure to PAHs. Also, we observed an important effect of mothers´ education level, when comparing university vs. other education.

5. CONCLUSIONS

We studied the impact of PAHs exposure in ambient air, determined in the ambient air as the concentration of benzo[a]pyrene (B[a]P) and OH-PAHs metabolites in urine of newborns in the time of delivery on cognitive development of 5 years old children, using Bender Visual Motor Gestalt Test (BG test) and the Raven Colored Progressive Matrices (RCPM test). We did not observe any effect of B[a]P exposure during the last trimester or OH-PAHs metabolites in the time of delivery to cognitive development in 5 years old children. Higher exposure to PM2.5 during the third trimester in Karvina decreased the results of BG test in girls in Karvina. We believe that given topic deserves further research.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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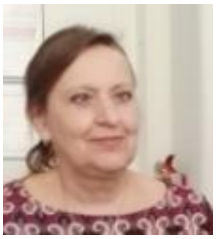

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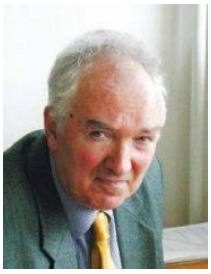

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Chapter 9
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Study about Tai Chi Chuan and its Benefits

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ABSTRACT

Tai Chi Chuan is an ancient Chinese body-mind practice. People practice it for different perspectives. The benefits and potential of Tai Chi Chuan for general health, alternative treatments for chronic diseases, anti- aging and social wellbeing, have being recognized by extensive research. Tai Chi Chuan is an art of living pursuit for a healthy, harmonious and happy life. As a lifestyle, Tai Chi performance is not just a simple exercise but an art of living. It deeply connects the Chinese cultures of Taoism, Confucianism and Traditional Chinese Medicine, among others.

Keywords: Tai chi chuan; yin and yang; internal martial art; moving meditation; alternative medicine; anti-aging solution; lifestyle.

1. WHAT IS TAI CHI CHUAN?

Tai Chi Chuan is a body and mind fitness regimen guided by Ancient Chinese wisdom - Yin and Yang, and Five Elements principles. It belongs to Chinese internal Martial Arts and has been practiced since 17th century. Tai chi combines gentle physical movement, mental imagery, and natural, relaxed breathing. There is increasing scientific evidence showing the impact of tai chi exercise on multifaceted areas of health and well-being, including positive effects on cognition, depression, anxiety, sleep, cardiovascular health, and fall prevention [1].

Historical records show that Yang Luchan (1799-1872), the creator of Yang Style Tai Chi Chuan, trained with the Chen family for approximately 18 years before he started to teach the art in Beijing. This strongly suggests that his art was based on, or heavily influenced by, the Chen family art. The Chen family can trace the development of their art back to Chen Wangting in the 17th century. Martial arts historian Xu Zhen believed that the Taiji of Chen Village had been influenced by the Taizu changquan style practiced at the nearby Shaolin Monastery, while Tang Hao, another martial arts historian, thought it was derived from a treatise by the Ming dynasty general Qi Jiguang, Jixiao Xinshu ("New Treatise on Military Efficiency"), written approximately 1561-1562, which discussed several martial arts styles including Taizu changquan [2].

There are five major styles of Tai Chi Chuan and each name of the style followed its creator's family:

- Chen style of Chen Wangting (1580–1660).
- Yang style of Yang Luchan (1799–1872).
- Wu Hao style of Wu Yu-hsiang (1812–1880).
- Wu style of Wu chuan-yu (1834–1902) and his son Wu Chien-chuan (1870–1942).
- Sun style of Sun Lu-t'ang (1861–1932) [2].

Initially people practiced Tai Chi Chuan for its martial purpose. By learning and practicing it, it was found that the entire body was strengthened and breathing became more regulated. Additional positive results included the calming of the mind, cultivating Qi, and lifting one's spirit. This encouraged more and more people to practice Tai Chi Chuan worldwide. More doctors and health professionals started recommending their patients practice Tai Chi. Tai Chi Chuan is the most

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prominent manifestation of tai chi in Chinese martial arts. Tai Chi Chuan has been reported to be potentially effective for health and well-being of both the sick and the healthy. However, it is still controversial whether it benefits breast cancer patients. It is therefore of great value to evaluate the effectiveness of Tai Chi Chuan on the psychological well-being and quality of life in people with breast cancer [3].

In 1956 Simplified 24 Yang Style Tai Chi Chuan was created and promoted nationally and then worldly by China Sports committee. It has gradually become a world renowned body- mind form of exercise which has led to numerous studies and clinical trials designed with one of two aims: either to assess physiological responses during the performance or to assess the impact of this exercise on general health and fitness.

1.1 Tai Chi Chuan in the United States

“Choy Hok Pang, a disciple of Yang Chengfu, was the first known proponent of tàijíquán to openly teach in the United States in 1939. Subsequently, his son and student Choy Kam Man emigrated to San Francisco from Hong Kong in 1949 to teach t‘ai-chi ch‘üan in San Francisco's Chinatown. Choy Kam Man taught until he died in 1994” [2]. In the United States there is growing awareness of the benefits of Tai Chi Chuan. According to statistic it shows the number of participants, aged six and older, practicing Tai Chi in the United States in 2015 had reached 3.65 million [4].

1.2 The Benefits of Tai Chi Chuan

The benefits of Tai Chi Chuan come from different perspectives:

As a fitness routine: It is considered a moderate intensity (doesn’t exceed 55% of maximal oxygen intake), low impact, aerobic exercise. It is not restricted by age, sex, space or time. Many studies show regular Tai Chi Chuan exercise promotes general health including cardio-respiratory and musculoskeletal function, posture control capacity, and the immune system [5]. When Tai Chi Chuan was compared to other moderate exercises performed at equal intensity, Tai Chi showed a significantly lower ventilatory equivalent (Ve/ Vo2MAX), meaning the exercise resulted in more efficient breathing than other moderate exercises [6].

The earliest medical research stared from 1959 by Beijing Medical University Institute of Sports Medicine. By using the one-minute step test, ECGs, and blood pressure, they examined 31 participants, age from 50-89, who had practiced Tai Chi Chuan for over 40 years. Compared with a control group “Tai Chi Chuan practitioners showed significantly better cardiac function and lower blood pressure” [7].

Some studies have focused on the impact of Tai Chi Chuan on immunological capacity on those without and with prior Tai Chi Chuan training. One study on those without prior training was done by Zhang [8] who studied the effect of Tai Chi on human immunoglobulin, IgA, IgG, IgM, and IgE, on thirty subjects (15 male, 15 Female, ages 50-65) after two months of Tai Chi Chuan training. The training included learning the 48 form for one hour of practice each day. Before the study was started baseline blood samples were drawn. Results after two months showed that Tai Chi significantly increased IgG in men and significantly decreased the level of IgM in woman, all within the normal range. This showed that Tai Chi may enhance the capacity of the immune system.

Another study by Li and Shen [9] studied changes in the number and activity of natural killer cells in peripheral blood of eight male Tai Chi Masters, average age 68-69, who had practiced Tai Chi Chuan every day for one to two hours for approx. nineteen years. For the study and after a twenty- minute Tai Chi session, blood samples were taken of each Master. It was found that the numbers and activity of natural killer cells in the peripheral blood had significantly increased. Also, this study found a concomitant increase in the level of cortisone in the blood. This indicates that Tai Chi Chuan may moderate the capacity of the immune system.

As an internal Martial Art, its philosophy is based on Yin and Yang principles “When the opponent is hard, I am soft”, “If the opponent’s is quick, then quickly respond; if his movement is slow. Then follow slowly” [10]. It emphasis on maintaining your balance meanwhile neutralizing the opponent’s force against you and destroying his or her balance.

As a sport, intense competition involves high level of skills, strength, balance, flexibility, coordination etc. Tai Chi athletes need comprehensive training to keep body, mind and energy level in greatest shape. It also has become the complement training for other sports. For example, Tom Brady, one of the greatest NFL quarterbacks in the history of the game, with five Super Bowl wins, practices Tai Chi. He practices it for his defense training and promotion of inner peace and to alleviate stress and anxiety [11]. Recently NBA‘s Stephen Curry has started incorporating Tai Chi into his pre-game routine with other teammates joining him as well [12].

As a meditation, the circular and rhythmic motion guided by focusing one’s mind leads the body into a meditative stage which people call “moving meditation”. It is apparent then that it will benefit the parasympathetic nerve system which helps reduce stress and improve concentration.

As complementary and alternative medicine, Tai Chi has six interdependent therapeutic elements which include:

- Tai Chi is a moderately aerobic exercise.
- Tai Chi trains agility and mobility.
- Tai Chi involves learning and memorization of new skills and movement patterns.
- Tai Chi includes training in sustained attentional focus, shifting, and multi-tasking.
- The meditative and relaxation training of Tai Chi.
- Greater time allocated to leisure activities and social support [13].

Existing science evidences has proven its benefit for people who suffer from chronic disease. “A recent report in the British Journal of Sports Medicine looked at 33 articles and 24 studies featuring nearly 1,600 participants. The report concluded that tai chi is an effective relief treatment for people suffering from breast cancer, heart failure, osteoarthritis, and chronic obstructive respiratory disease (COPD). Furthermore, it was found to help people with one or more of these conditions and did not cause any additional pain or breathlessness” [14]. Medical school offer Tai Chi Chuan class to students as a requirement is trendy.

As Anti – aging solution, the growing evidence shows that Tai Chi Chuan can help slow down the aging process. A new study found that those who practiced Tai Chi enjoyed a significantly higher number of CD34 cells than those in the other groups. CD34+, known as the stem cells, is “markers for hematopoietic stem cells (blood stem cells) involved in cell self-renewal, differentiation and proliferation” [15]. Other evidence indicates “Tai Chi may be an effective, safe, and practical intervention for maintaining BMD in postmenopausal women” and can positively impact other risk factors associated with low BMD such as reduced fall frequency, increased musculoskeletal strength and reduced fracture risk [16] Also Tai Chi may afford greater effectiveness in enhancing cognitive function based on its six interdependent therapeutic elements [13].

When people get older, many with less physical exercise options can choose Tai Chi as a safe lifelong practice offering tremendous benefits in many ways.

As a lifestyle, Tai Chi performance is not just a simple exercise but an art of living. It deeply connects the Chinese cultures of Taoism, Confucianism and Traditional Chinese Medicine, among others. By understanding and applying the Yin-Yang principle we pursuit a life with balance, inner peace and happiness as well as longevity.

2. CONCLUSION

Tai Chi Chuan is an ancient Chinese body-mind practice. It came to the United States in 1939. People practice it for different perspectives. The benefits and potential of Tai Chi Chuan for general health,

alternative treatments for chronic diseases, anti- aging and social wellbeing, have being recognized by extensive research. Tai Chi Chuan is an art of living pursuit for a healthy, harmonious and happy life.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Chapter 10
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The Use of Free Thyroxine and Free Triidothyronine as an Index for the Assessment of Thyroid Function in Port Harcourt, Rivers State, Nigeria

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ABSTRACT

A critical part of diagnosing thyroid disorders is the laboratory evaluation performed through blood testing. A reliable and accurate diagnosis is necessary in order to select the proper treatment options for thyroid disorder patients and help to increase workflow and efficiency. This research was carried out because of increased late diagnosis of thyroid disease in patients suffering from various thyroid disorders. Therefore the purpose of this chapter is to present concepts to practical diagnosis of thyroid diseases.

Objective: The objective of this study was to establish the usefulness of fT3 and fT4 in the diagnosis of different thyroid diseases. The medical personnel treating thyroid disorders need the appropriate ammunition to approach their patient's problems. The estimation of free thyroxine, fT_4, and free triodothyronine, fT_3 was done and used as an index for the differential assessment of thyroid function. A total number of nine hundred and seven (907) patients were diagnosed of various thyroid dysfunctions from the subjects that attended the various hospitals/clinics in the city of Port Harcourt, Rivers State, Nigeria, between the months of February, 2010 to April, 2013. The method of enzyme linked immuno sorbent assay (ELISA) was used in carrying out the study. Out of the 907 patients, 532 of them, representing 58.65% were females while 375 of the patients representing 41.35% were males. From the result analysis, 55.46% of the patients diagnosed of primary hyperthroidism had elevated serum levels of both total and free T_3, T_4 with low TSH values, while 3.09% of the hyperthyroid cases had normal total T_4 and T_3 but elevated values of fT_4 and fT_3. 0.88% of the patients with hypothyroidism had normal total T_4 and T_3, but with low serum levels of fT_4 and fT_3 and was diagnosed so clinically. Triiodothyronine (T_3) toxicosis with elevated fT_3 and normal fT_4 serum levels were found in 9.81% of the patients. Non thyroidal illness cases had low serum fT_4 in 9.26% of the patients. Two (about 0.22%) of the patients that were diagnosed of TSH Secreting tumours had an elevated serum fT_4 level in addition to high serum TSH levels. This study showed that fT_3 and fT_4 is actually an index in the differential diagnosis of thyroid diseases.

Keywords: Hyperthyroidism; Hypothyroidism; Toxicosis; Non –Thyroidal; Hormone; Free Thyroxine; Free Triidothyronine.

1. INTRODUCTION

The sequence of events leading to the development of the thyroid and the control and secretion of the thyroid hormones in humans has been well reviewed [1]. At birth, an acute release of thyrotrophin (TSH) occurs which stimulates an increase of both thyroxine (T4) and triiodothyronine (T3) concentrations in serum [2]. Thyroxine (T_4), tri-iodothyronine (T_3) and calcitonin are secreted by the thyroid gland. The T_4 and T_3 are products of the follicular cells and generally influence the rate of all metabolic processes. The hormones are synthesized in the thyroid gland by the iodination and

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coupling molecules of the amino acid tyrosine, a process that is dependent on an adequate supply of iodide [3]. Iodide is actively taken up by the thyroid gland under the control of thyroid stimulating hormone (TSH) via a sodium/iodide symporter. Uptake is blocked by thyiocyanate and perchlorate. The concentration of iodide in the gland is at least 20 times that in plasma and may exceed it by 100 times more [4]. Secretion of the thyroid hormones is regulated by pituitary thyrotropin (TSH). TSH secretion in turn is controlled through negative feedback by the thyroid hormones. There is a negative log-linear relationship between serum free T_4 and TSH concentrations [5].

Most of the plasma T_4 and T_3 is protein bound mainly (70 %) to an α-globulin, thyroxine binding globulin (TBG) and to a lesser extent (15.0 %), transthyretin with about 10-15 % bound to albumin and thyroxine binding pre albumin (TBPA). Crook, in 2001 stated that in keeping with many other hormones; the free unbound fraction is the physiologically active form, which also regulates TSH secretion from the anterior pituitary. This means that very small changes in serum free T_4 concentrations induce very large reciprocal changes in serum TSH concentrations. As a result, thyroid function is best assessed by measuring serum TSH, assuming steady state conditions and the absence of pituitary or hypothalamic disease [6].

Some of the circulating T_4 is de-iodinated by enzymes in peripheral tissues, especially in the liver and kidneys. About 80 % of the plasma T_3 is produced by the removal of an iodine atom from the outer (β) ring, the remaining 20 % is secreted by the thyroid gland. De-iodination of the inner (α) ring produces reverse T_3, which is probably inactive [7].

The thyroid hormones generally affect many metabolic processes, increasing oxygen consumption. They bind to specific receptors in cell nuclei and change the expression of certain genes [8]. Thyroid hormones are essential for normal growth, mental development and sexual maturation and also increase the sensitivity of the cardiovascular and central nervous systems to catecholamine's, thereby influencing cardiac output and heart rate [9].

Assessment of thyroid hormone secretion can be made by measuring plasma TSH as well as either fT_4 or total T_4 (same as fT_3 or total T_3). Each test has its advantages and disadvantages and many laboratory thyroid assays can now measure fT_4 and fT_3 rather than total hormone concentrations [10]. Plasma T_4 is more than 99 % protein bound, therefore, plasma, Total T_4 assays reflect the protein bound rather than the free hormone fraction. Total T_4 reflects fT_4 concentrations unless there are abnormalities of binding proteins. This also is applicable to plasma T_3 or fT_3 concentrations [11]. The thyroid hormones also regulate protein, fat and carbohydrate metabolism, affecting how human cells use energetic compounds. They also stimulate vitamin metabolism and therefore numerous physiological and pathological stimuli influencing thyroid hormone synthesis.

Thyroid function tests are blood tests used to evaluate how effectively the thyroid gland is working. The tests are ordered and indeed interpreted by the physician and it includes thyroid stimulating hormone (TSH) free and total thyroxine (fT_4, T_4) the free and total triiodothyronine (fT_3, T_3) depending on local laboratory policy. The thyroxine binding globulin (TBG) and the T_3 uptake tests are used to diagnose underactive thyroid (hypothyroidism) and over active thyroid (hyperthyroidism), evaluate thyroid gland activity and monitor response to thyroid therapy. A doctor may order a free T3, T4 test if a patient is having symptoms of a thyroid disease such as weight loss, a rapid heart rate, and sweating associated with hyperthyroidism or vice vasa in hypothyroidism [12]. This study was undertaken to assess the use fullness of fT_4 and fT_3 in the differential diagnosis of thyroid function.

2. MATERIALS AND METHODS

2.1 Study Area/ Population

This study was carried out in Port Harcourt, Rivers State, South of Nigeria. The subjects consisted of nine hundred and seven (907) patients, male and female diagnosed of various thyroid disorders that attended the different hospitals/clinics in the city of Port Harcourt, Rivers State, Nigeria. The figure breakdown showed a total of three hundred and seventy five (41.35%) males of different age range and five hundred and thirty two (58.65%) females also of different age range were studied.

2.2 Sample Collection and Preparation

About 10 ml of venous blood were collected from the patients in the different centres using the standard vein puncture technique and its after obtaining a consent from the patient and the centre management. This was discharged into a plain tube without additives and allowed to clot. The serum from the sample was separated after centrifugation at 3,000 rpm and stored frozen at -20^{o}C. Analyses of the samples were done within 7 days of collection.

2.3 Assay Method

The method of enzyme linked immunosorbent assay (ELISA) was used in the quantitation of the various thyroid hormones. The ELISA test is based on the principle of solid phase enzyme linked immunosorbent techniques, where the antibody to be measured is incubated with specific antigen coupled to a solid phase [14,15]. This is a quantitative determination of free T3, free T4; Thyrotropin, TSH and other analytes for a comprehensive thyroid status of a human serum or plasma sample by a Microplate Enzyme Immunoassay. Measurements of thyroid hormones (fT3, fT4, TSH) are generally regarded as invaluable in-vitro diagnostic tests for assessing thyroid function [16]. The importance has provided the need for the significant improvement in assay methodology that has occurred in the last three decades. This procedural evolution is traced from empirical protein bound iodine (PBI) test [17] to the theoretically sophisticated radioimmunoassay and currently used EIA, ELISA. FIA and Chemiluminescence.

2.4 Statistical Analysis

The percentage tool was used in the analysis of the data.

3. RESULT

Table 1. Free thyroxine and triidothyronine as index for the assessment of thyroid function in Port Harcourt

Hormone parameter assayed

Type of thyroid disorder	Percentage	No of Patients	TT_3	TT_4	FT_3	FT_4	TSH	TBG
Primary hyperthyroidism	55.46%	503	↑	↑	↑	↑	↓	Normal
Secondary hyperthyroidism	0.88%	8	↑	↑	↑	↑	↑	Normal
Primary hypothyroidism	15.44%	140	↓	↓	↓	↓	↑	Normal
Secondary hypothyroidism	0.22%	2	↓	↓	↓	↓	↓	Normal
T_3 toxicities	9.81%	89	↑	Normal	↑	Normal	↓	Normal
TBG excess	1.21%	11	↑	↑	Normal	↓	Normal	↑
TBG deficiency	0.55%	5	↓	↓	Normal	↑	Normal	↓
Non thyroidal illness	9.26%	84	Normal	Normal	Normal	↓	Normal	Normal
Subclinical hypothyroidism	1.10%	10	Normal	Normal	Normal	Normal	↓	Normal
TSH-Secreting tumour	0.22%	2	Normal	Normal	Normal	↑	↑	Normal
Euthyroid patient with cancer/goiter	1.88%	17	Normal	Normal	Normal	Normal	↑	Normal
*Hyperthyroid	3.09%	28	Normal	Normal	↑	↑	↓	Normal
*Hypothyroid	0.88%	8	Normal	Normal	↓	↓	↑	normal

Legend: ↑: increase, ↓: decrease

**Hyperthyroid refers to the patient diagnosed of hyperthyroidism but had normal total T_3 and normal total T_4 but with elevated free forms*

**Hypothyroid refers to the percentage of patients diagnosed of hypothyroidism with normal total T_3 and T_4 but with decreased serum free forms*

4. DISCUSSION

The free thyroxine (fT_4) and free triiodothyronine (fT_3) tests, commonly done simultaneously measure serum levels of fT_4 and fT_3, which is the minute portions of total T_4 and T_3 not bound to thyroxine binding globulin (TBG) and other serum proteins. These unbound hormones are responsible for the thyroids effect on cellular metabolism. Because of disagreement as to whether fT_4 or fT_3 is the better indicator, both are commonly measured in laboratory. The disadvantages of these tests include complex laboratory method, cost and its inaccessibility. The test is very useful in some patients in whom the standard total T_3 and T_4 test fail to produce diagnostic results [18] particularly when the state of the pituitary or hypothalamic function is required [19].

The evidence that fT_4 and fT_3 are better indicator for the diagnosis of various thyroid dysfunctions was shown in this study were fT_4 and fT_3 values actually confirmed the patient diagnosis. From the result, 503 patients diagnosed of primary hyperthyroidism had elevated serum levels both in total and free T_4 and T_3 and low TSH values. While the rest 28 hyperthyroid patients had normal total T_4 and T_3 but elevated values for fT_4 and fT_3. Clinical presentations of the patients, agreed with these laboratory findings.

These were also applicable to the 8 patients diagnosed of hypothyroidism with normal total T_4 and T_3 but low serum levels of fT_4 and fT_3, and were clinically hypothyroid by this study. There were generally fT_4 involvements in the diagnosis of the other thyroid disorders. Eighty nine (89) patients diagnosed of T_3 toxicosis had normal fT_4 serum levels, eighty four (84) patients diagnosed of non-thyroidal illness had a low serum fT_4 levels and two (2) patients diagnosed of TSH secreting tumours had an elevated serum fT_4 levels in addition to high serum TSH values. These were the findings from the thyroid assay done in this locality between the months of February, 2010 and April, 2013.

It's argued in some quarters that the high sensitivity thyroid stimulating hormones (TSH) test is the most sensitive and specific screening test for thyroid disease [20]. A first line test for thyroid function usually, is plasma TSH, which should not be interpreted in the absence of plasma fT4 and sometimes fT3 is also required, particularly if hyperthyroidism is suspected. That TSH levels change exponentially with changes in T_4 and T_3 and are less likely to be elevated or depressed by non-thyroidal illness or drugs. They concluded that the strategy is more cost-effective than a panel approach of TSH + fT_4 and TT_4 or fT_3 and TT_4. Its worthy of note that values of TSH below 0.02 miu/L requires differential diagnosis of primary hyperthyroidism which causes levels to be near undetectable from the low end of the reference range, which is only 0.4 miu/L and that normal TSH levels rules out clinical thyroid disease [21]. This means that low TSH levels might be as a result of primary hyperthyroidism or secondary hypothyroidism caused by the pituitary TSH deficiency. High TSH levels are caused by the pituitary TSH deficiency. High TSH level are caused by hypothyroidism or secondary hyperthyroidism resulting from pituitary adenoma. These situations therefore require (and are normally followed by) measurement of free T_4 and free T_3 to confirm the diagnosis. From the study, a patient with low TSH who has primary hyperthyroidism had an elevated T_3 and also elevated free T_4 or T_3 , a patient with a low serum TSH values caused by pituitary disease had low levels of these hormones [22].

5. CONCLUSION

It can be concluded therefore that measurement of free T_4 and free T_3 is more sensitive and better specific indicator in the diagnosis of thyroid diseases. However, there remain a few situations, according to Dayan (2001), in which the results of TSH, free T_4 and free T_3 assays tend to point in different directions, as well as cases in whom thyroid function test results seem clear cut but are in fact misleading. The challenge lies in applying them to the right individual at the right time.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 11
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Relationship of Clinical Data and Confirmed Case of COVID-19, in the Mexican State of Guanajuato

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ABSTRACT

Aims: The objective was to analyze the clinical data in the population from Mexican Guanajuato state as a suspected case of COVID-19 and with result positive of rRT-PCR, reported until October 2, 2020.
Intorduction: Since the beginning of the new coronavirus pandemic in Wuhan, China, at the end of 2019 , one of the concerns has been to have a precise diagnosis. Fever is the most reported sign in COVID-19 cases , but it is also present in other viral infections.
Study Design: It is a cross-sectional study based on the data from the National Epidemiological Surveillance System of the General Epidemiological Directorate, from the Mexican Secretary of Health.
Place and Duration of Study: Sample registries from confirmed and discarded cases of COVID-19 in the database until October 2, 2020.
Methodology: 100,919 registries were considered. Among them, 810 were excluded due to the absence of the result of rRT-PCR test. A suspected case was one with a clinical finding considered greater (cough, fever, headache, or dyspnea and accompanied by at least one of the following: myalgia, arthralgia, odynophagia, chills, chest pain, rhinorrhea, anosmia, dysgeusia, or conjunctivitis); a confirmed case of COVID-19 is a person with a positive rRT-PCR test for SARS-CoV-2, regardless of the clinical data presented. We included age, sex, and clinical data registered and the result of rRT-PCR for SARS-CoV-2. It was used logistic regression to analyze the effect of clinical data on positive rRT-PCR.
Results: It was analyzed 100,109 registries. From them, 41,734 were positive for SARS-CoV-2. Fever (OR 1.72, CI95% 1.68 to 1.77), cough (OR 1.70, CI95% 1.66 to 1.74), and odynophagia (OR 1.71, CI95% 1.66 to 1.75) shown a stronger effect on positive rRT-PCR test. Cyanosis did not have any effect on the result of the rRT-PCR test.
Conclusion: There are no pathognomonic clinical data for COVID-19. All clinical data in confirmed cases are like other respiratory viral infections.

Keywords: SARS-CoV-2; COVID-19; clinical data; confirmed case.

1. INTRODUCTION

Since the beginning of the new coronavirus pandemic in Wuhan, China, at the end of 2019 [1], one of the concerns has been to have a precise diagnosis. In Mexico, in April 2020, the definition of a suspected case was any person of any age who in the last seven days has presented at least two of the following signs and symptoms: cough, fever, or headache, accompanied by at least one of the following signs or symptoms: dyspnea (severity data), arthralgia, myalgia, odynophagia/pharyngeal burning, rhinorrhea, conjunctivitis, thoracic pain [2], and a confirmed case is any person who meets the operational definition of a suspected case and has with a laboratory-confirmed diagnosis of the National Network of Public Health Laboratories recognized by the Epidemiological Reference Institute [2].

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With the increasing number of cases from COVID-19 in Mexico, the criteria for a suspicious case of viral infectious disease changed to the following: any person with fever, headache, dyspnea, or cough, more one of the following symptoms: myalgias, arthralgias, odynophagia, chills, thoracic pain, rhinorrhea, anosmia, dysgeusia, conjunctivitis [3].

Faced with the imminent arrival of the pandemic to the state of Guanajuato, the state civil authorities took preventive actions such as social distance orders, staying at home, and closure of educational institutions of all levels from March 20, 2020. A week later, restaurants, gyms, and public parks were closed. Also, massive events and meetings between more than ten persons were canceled. These measures contained the spread of the infection in the state. Nevertheless, the number of confirmed cases increased later.

In Guanajuato state, the first confirmed case of the disease by the new coronavirus (COVID-19) was confirmed on March 10, 2020 [4]. Several papers show the evolution in the number of confirmed cases of COVID-19; as of March 23, only 12 cases had been confirmed [5], 37 confirmed cases were reported by March 28 [6], 96 by April 16 [7], 658 by May 15 [8], and 838 by May 20 [9], giving an increase from March 23 to May 20 of 698.33%.

2. GUANAJUATO STATE

The state of Guanajuato is in the center of the Mexican Republic, with location data: Longitude #102°5'49.2 “W #99°40'16.68” W, Latitude 19°54'46.08 "N 21°50'21.84 ”N [10]. The state had 5,486,372 inhabitants, which represented 4.88% of the Mexican population, according to the 2010 national census [11].

The World Health Organization (WHO), in the document Diagnostic Tests for SARS-CoV-2: Interim Guidance, recommends real-time reverse transcription-polymerase (rRT-PCR) for the diagnosis of SARS-CoV-2 infection and COVID-19 [12].

Guanajuato State was the first Mexican state with a hospital exclusively for COVID-19 patients [13].

2.1 National Epidemiological Surveillance System (NESS) from General Directorate of Epidemiology (GDE) from the Secretary of Health (SH) from Mexico

The NESS/GDE database is an official and open database from the Secretary of Health from the Mexican Government, where all the cases under investigation are registered cumulatively. This database includes those suspected, confirmed, and negative test result cases to COVID-19 [14].

3. INSTITUTE OF PUBLIC HEALTH FROM GUANAJUATO STATE

The Government of the State of Guanajuato, committed to face the pandemic by SARS-CoV-2 and COVID-19, by supporting Guanajuato families through the Secretary of Health of the State of Guanajuato (SHSG); the Institute of Public Health from Guanajuato State (IPHGS) has the resources and generation of epidemiological information, maintaining communication with the population of the state [13].

Due to the declaration of the pandemic by the World Health Organization in March 2020, the first confirmed case in Guanajuato (March 2020), and the first death in the same state (April 2020), the five essential measures to prevent the spread of SARS-CoV-2 were broadcasted: hand washing, use of face masks, social distancing, cleaning surfaces and isolation at home [13].

The SHSG has 53 mobile units, ensuring access to primary health care services, focusing on the SARS-CoV-2 virus; IPHGS has 44 hospitalization units (15 general hospitals, 20 community hospitals, eight specialized hospitals, one mobile hospital, in addition to 583 first-level care units, the State Public Health Laboratory, the Medical Emergency System, and a State Medical Center. Transfusion. The 44 hospitalization units have a respiratory triage system for classifying patients, performing tests,

diagnosing COVID-19, and determining their management: hospitalization or outpatient treatment [13].

In March 2020, the old General Hospital of León was renovated, and it became the State Hospital for COVID-19 Care. In addition, there is the Salamanca State Center for Critical Care. Both hospitals were exclusively dedicated to COVID-19 patients. Later, the Mobile Hospital was purchased by the state for the care of COVID-19 patients [13].

Currently, ISAPEG has 1,797 beds for the care of patients with COVID-19 and severe acute respiratory infection [13].

4. CLINICAL DATA OF COVID-19

Fever is the most reported sign in COVID-19 cases [15], but it is also present in other viral infections [16].

In three series of COVID-19 patients in China, fever and cough were the most frequent in COVID-19 patients of all ages, following dyspnea, myoarthralgia, sore throat, headache, rhinorrhea, vomiting, and diarrhea [17-19].

Clinical data as loss of smell and taste have been associated with COVID-19, and they are more common in COVID-19 (39%) than in influenza (13%) [20]. Cough is present in 58% patients with COVID-19 [15].

Dyspnea, shore throat, rhinorrhea, myalgias, arthralgias, vomit, diarrhea, or fatigue are present in COVID-19 as in other viral infections [21].

In the state of Guanajuato, in May 2020, in COVID-19 patients of all ages, fever, cough, sore throat, headache and dyspnea were reported as the most frequent; in addition, vomiting and diarrhea, chest pain, abdominal pain, rhinorrhea, myoarthralgia rhinorrhea were reported [9].

5. MATERIALS AND METHODS

It is a cross-sectional analytical study of clinical data and the result of the rRT-PCR test, registered in the database of the SINAVE/DGE, of confirmed and discarded cases of COVID-19 [22,23]. The database was used until October 2, 2020, including the confirmed and discarded cases of COVID-19 in Mexico, which were registered according to the Sentinel model.

All the records that had complete data were selected for the analysis. There were no exclusion criteria, and the elimination criteria were records with missing data.

For the SINAVE/DGE, a suspected case was one with a clinical finding considered major (cough, fever, headache, or dyspnea), accompanied by at least one of the following: myalgia, arthralgia, odynophagia, chills, chest pain, rhinorrhea, anosmia, dysgeusia or conjunctivitis [13].

A confirmed case of COVID-19 is a person with a positive rRT-PCR test result for SARS-CoV-2, and clinical data as cough, fever, dyspnea, headache [14].

The sociodemographic variables age and sex were included in the analysis. The variables considered as independent were the onset of clinical data, fever, cough, headache, dyspnea, shore throat, irritability, diarrhea, thoracic pain, chills, myalgia, arthralgia, malaise, rhinorrhea, polypnea, vomit, abdominal pain, conjunctivitis, cyanosis, anosmia, and dysgeusia.

The dependent variable was the result of the rRT-PCR test, applied to the sample taken from the nasal and oropharyngeal mucosa. For this analysis, the registries where a positive rRT-PCR test result for respiratory viruses distinct to SARS-CoV-2 is specified were considered as negative to the presence of SARS-CoV-2.

5.1 Procedures

The Excell ® database was reviewed and it was transferred to the STATA 13.0 database (Stata Corp., College Station, TX, USA).

5.2 Statistical Analysis

Descriptive statistics are presented for all variables, and an epidemiological curve was designed for confirmed cases according to the onset of symptoms. Tabulation of each clinical characteristic was performed for confirmed cases and discarded cases. The Chi-square test, the corresponding degrees of freedom, and the P-value were calculated to show an association between the variables and the rRT-PCR test result. Odds Ratio (OR) and their corresponding 95% confidence intervals (95% CI) were calculated to show the effect of the clinical characteristics on being a confirmed case.

A logistic regression model was fitted, and it was determined whether age group or sex acted as confounders, with the Likelihood Ratio Test (LRT) and the corresponding P-value. Logistic regression models were fitted including all clinical characteristics, and the models were compared with the LRT and the P-value.

In all cases, to demonstrate statistical significance, the alpha value was set at .05. Statistical analysis was performed in STATA 13.0 (Stata Corp., College Station, TX, USA).

6. RESULTS AND DISCUSSION

100,919 records were obtained up to October 2, 2020, from the SINAVE/DGE database [4]. 810 records were eliminated due to the rRT-PCR test result absence, leaving 100,109 records for further analysis.

From the 100,109 suspected cases up to October 2, 2020, 41.69% were positive for SARS-CoV-2 and a few for other respiratory viruses (Table 1).

Table 1. Distribution of results of rRT-PCR test in suspected cases of COVID-19 (n=100,109)

Results of RT-PCR test	n	%
Negative	58,086	58.02
SARS-CoV-2	41,734	41.69
A H1N1	147	0.15
Influenza B	110	0.11
A H3	12	0.01
Respiratory Syncytial Virus	10	0.01
Rhinovirus	3	0.00
Influenza A	3	0.00
Metapneumovirus	3	0.00
Parainfluenza 2	1	0.00

Source: SINAVE/DGE [4]

By the onset of symptoms, the curve of confirmed data is obtained (Fig. 1), where it is verified that the highest number of cases was on July 25, 2020, with 676 new confirmed cases and, although they have decreased, there have been between 300 and 400 new cases per day during August 2020. In September, we would have to wait for the results of the rRT-PCR test to corroborate whether it is a bonafide decrease or is an artifact due to the lack of laboratory test results.

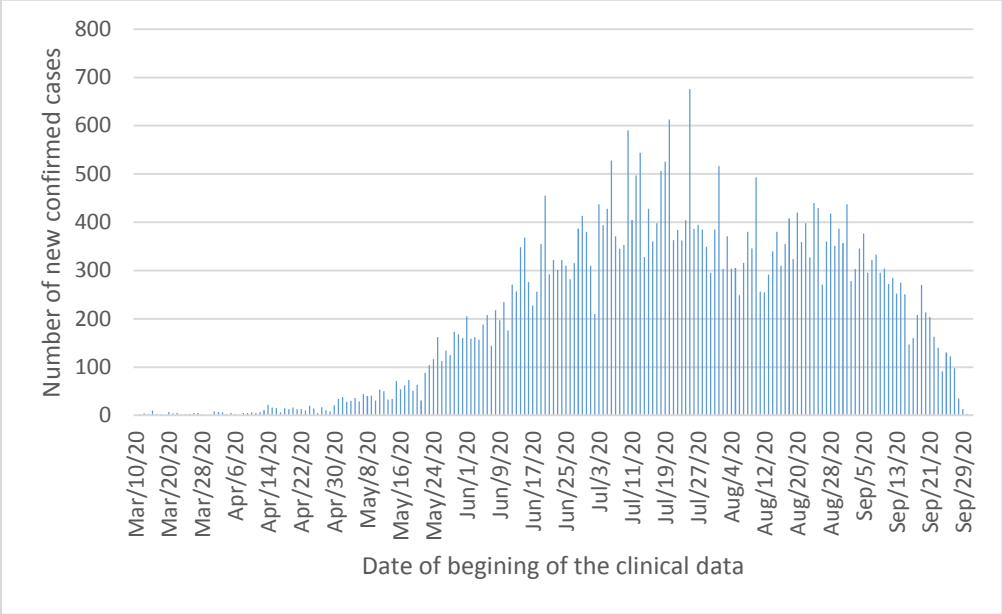

Fig. 1. Epidemiological curve of confirmed cases until October 2, 2020 (n=41,734)
Source: SINAVE/DGE [4]

From the records, it was obtained that 53,396 (53.34%) were women and 46,713 (46.66%) men, with ages ranging from 0 to 113 years, and a mean age of 39.44 ± 17.58.

Among the records of people with a positive rRT-PCR test result, the age group between 12 and 49 years (63.77%) and women (51.38%) predominated. Among the registries with a negative rRT-PCR test result, the same age range from 12 to 49 years (70.67%) and women (54.74%) prevailed (Table 2).

Table 2. Distribution of results of rRT-PCR positive to SARS-CoV-2 by age group and sex (n=100,109)

Age group (years)	rRT-PCR test positive		rRT-PCR test negative		X^2-test (df) *P*-value
	n	%	n	%	
Age group (years)					2,100 (6) .0001
0 – 2	273	0.65	1,121	1.92	
3 – 5	262	0.63	913	1.56	
6 – 11	553	1.33	1,827	3.13	
12 – 49	26,614	63.77	41,251	70.67	
50 – 59	6,715	16.09	6,901	11.82	
60 -74	5,504	13.19	4,772	8.17	
75 -115	1,813	4.34	1,590	2.72	
Sex					109.94 (1) .0001
Female	21,444	51.38	31,952	54.74	
Male	20,290	48.62	26,423	45.26	

Source: SINAVE/DGE [4]

As expected, 15.44% were hospitalized among the confirmed cases, approximately twice that in the discarded cases (6.84%) (Table 3).

Table 3. Distribution of type of patient by result for SARS-CoV-2 of rRT-PCR test

	rRT-PCR test positive (n=41,734)		rRT-PCR test negative (n=58,375)		Z-test for two proportions and *P*-value
	n	%	n	%	
Hospitalized	6,445	15.44	3,990	6.84	165.68 .00001
Ambulatory	35,289	84.56	54,385	93.16	

Source: SINAVE/DGE [12]

Of the records excluded from the analysis, only anosmia and dysgeusia exceeded 4% of the records; the rest did not reach 1% (Table 4).

Table 4. Registries eliminated by not full information

Clinical data	rRT-PCR positive		rRT-PCR negative	
	n	%	n	%
Fever	16	0.04	17	0.03
Cough	7	0.02	13	0.02
Headache	11	0.03	28	0.04
Dyspnea	5	0.01	11	0.02
Shore throat	11	0.03	38	0.07
Irritability	4	0.01	11	0.02
Diarrhea	2	0.00	8	0.01
Thoracic pain	7	0.02	35	0.06
Chills	9	0.02	35	0.06
Myalgia	14	0.03	36	0.06
Arthralgia	13	0.03	31	0.05
Attack on the general state	3	0.01	13	0.02
Rhinorrhea	7	0.02	26	0.04
Polypnea	0	0.00	29	0.05
Vomit	3	0.01	26	0.04
Abdominal pain	7	0.02	32	0.05
Conjunctivitis	7	0.02	23	0.04
Cyanosis	5	0.01	27	0.05
Anosmia	1,951	4.67	9,743	16.69
Dysgeusia	1,970	4.72	9,768	16.73

Source: SINAVE/DGE [4]

All the clinical data reported between confirmed and discarded cases are shown in Table 5. Headache 83.03%, cough 81.41%, myalgia 64.28%, fever 66.22%, dysgeusia 63.90%, arthralgia 57.42%, odynophagia 56.80%, predominated.

Table 5. Distribution of clinical data by results of rRT-PCR test (n=100,109)

Clinical data	rRT-PCR test positive		rRT-PCR test negative		X^2-test (degree of freedom) P-value
	n	%	n	%	
Fever					1,900 (1) .0001
Yes	27,624	66.22	30,688	52.59	
No	14,094	33.78	27,670	47.41	
Cough					455.28 (1) .0001
Yes	33,969	81.41	44,210	75.75	
No	7,758	18.59	14,152	24.25	
Headache					0.0002 (1) .99
Yes	34,656	83.06	48,465	83.06	
No	7,067	16.94	9,885	16.94	

Clinical data	rRT-PCR test positive		rRT-PCR test negative		X^2-test (degree of freedom) P-value
	n	%	n	%	
Dyspnea					1,900 (1) .0001
Yes	9,250	22.17	6,880	11.79	
No	32,479	77.83	51,484	88.21	
Odynophagia					18.08 (1) .0001
Yes	22,866	54.80	32,777	56.19	
No	18,857	45.20	25,560	43.81	
Irritability					47.36 (1) .0001
Yes	4,566	10.94	5,608	9.61	
No	37,164	89.06	52,756	90.39	
Diarrhea					139.92 (1) .0001
Yes	7,795	18.68	9,239	15.83	
No	33,937	81.32	49,128	84.17	
Thoracic pain					1,300 (1) .0001
Yes	9,521	22.82	8,091	13.87	
No	32,206	77.18	50,249	86.13	
Chills					1,000 (1) .0001
Yes	16,890	40.48	17,926	30.73	
No	24,835	59.52	40,414	69.27	
Myalgia					709.30 (1) .0001
Yes	26,817	64.28	32,607	55.89	
No	14,903	35.72	25,732	44.11	
Arthralgia					1,000 (1) .0001
Yes	23,956	57.42	27,576	47.26	
No	17,767	42.58	30,768	52.74	
Attack on the general state					1,400 (1) .0001
Yes	18,545	44.44	19,075	32.68	
No	23,186	55.56	39,287	67.32	
Rhinorrhea					45.99 (1) .0001
Yes	13,988	33.52	20,768	35.59	
No	27,739	66.48	37,581	64.41	
Polypnea					1,200 (1) .0001
Yes	4,857	11.61	3.287	5.63	
No	36,897	88.39	55,059	94.37	
Vomit					110.28 (1) .0001
Yes	3,265	7.82	3,574	6.13	
No	38,466	92.18	54,775	93.87	
Abdominal pain					42.39 (1) .0001
Yes	4,631	11.10	5,733	9.83	
No	37,096	88.90	52,610	90.17	
Conjunctivitis					13.71 (1) .0001
Yes	4,287	10.27	5,582	9.57	
No	37,440	89.73	52,770	90.43	
Cyanosis					311.04 (1) .0001
Yes	1,406	3.37	963	1.65	
No	40,323	96.63	57,385	98.35	
Anosmia					2,300 (1) .0001
Yes	8,756	22.01	4,971	10.22	
No	31,027	77.99	43,661	89.78	
Dysgeusia					2,200 (1) .0001
Yes	8,260	63.90	31,504	41.76	
No	4,666	36.10	43,941	58.24	

Source: SINAVE/DGE [4]

Logistic regression models were fitted to identify the effect of clinical data on having a positive rRT-PCR test result, assessing each sign and symptom reported by confirmed cases (Table 6).

The final model included all clinical data, except cyanosis, as the model did not improve by considering this variable (P> .05).

The final model shows that fever, cough, dyspnea, chest pain, chills, myalgia, arthralgia, malaise influenced having a positive rRT-PCR test result for SARS-CoV-2. On the other hand, odynophagia, irritability, headache, rhinorrhea, abdominal pain, conjunctivitis, anosmia, and dysgeusia had a preventive effect on having a positive rRT-PCR test result for SARS-CoV-2. Diarrhea and vomiting did not show any effect on having positive rRT-PCR, after adjusting for the rest of the clinical data.

Table 6. Models of Logistic regression of clinical data and rRT-PCR test positive

Models	OR	CI95%	Likelihood Ratio Test	*P*-Value
rRT-PCR+ and fever	1.72	1.68 to 1.77	1,773.35	.00001
rRT-PCR +, fever and cough	1.70	1.66 to 1.74	309.20	.00001
rRT-PCR+, fever, cough, and odynophagia	1.71	1.66 to 1.75	101.85	.00001
rRT-PCR +, fever, cough, odynophagia, and dyspnea	1.61	1.57 to 1.65	1,264.19	.00001
rRT-PCR +, fever, cough, odynophagia, dyspnea and irritability	1.61	1.57 to 1.65	5.89	.02
rRT-PCR +, fever, cough, odynophagia, dyspnea, irritability and diarrhea	1.60	1.56 to 1.64	33.70	.00001
rRT-PCR +, fever, cough, odynophagia, dyspnea, irritability, diarrhea and thoracic pain	1.58	1.54 to 1.62	290.75	.00001
rRT-PCR +, fever, cough, odynophagia, dyspnea, irritability, diarrhea, thoracic pain and chills	1.52	1.48 to 1.57	231.79	.00001
rRT-PCR +, fever, cough, odynophagia, dyspnea, irritability, diarrhea, thoracic pain, chills and headache	1.52	1.48 to 1.56	8.63	.003
rRT-PCR +, fever, cough, odynophagia, dyspnea, irritability, diarrhea, thoracic pain, chills, headache and myalgia	1.48	1.44 to 1.52	144.12	.00001
rRT-PCR +, fever, cough, odynophagia, dyspnea, irritability, diarrhea, thoracic pain, chills, headache, myalgia and arthralgia	1.46	1.42 to 1.50	83.18	.0000
rRT-PCR +, fever, cough, odynophagia, dyspnea, irritability, diarrhea, thoracic pain, chills, headache, myalgia, arthralgia and attack on the general state	1.43	1.39 to 1.47	233.17	.00001
rRT-PCR +, fever, cough, odynophagia, dyspnea, irritability, diarrhea, thoracic pain, chills, headache, myalgia, arthralgia, attack on the general state and rhinorrhea	1.43	1.39 to 1.47	94.61	.00001
rRT-PCR +, fever, cough, odynophagia, dyspnea, irritability, diarrhea, thoracic pain, chills, headache, myalgia, arthralgia, attack on the general state, rhinorrhea and polypnea	1.43	1.39 to 1.47	29.87	.00001
rRT-PCR +, fever, cough, odynophagia, dyspnea, irritability, diarrhea, thoracic pain, chills, headache, myalgia, arthralgia, attack on the general state, rhinorrhea, polypnea and vomit	1.43	1.39 to 1.47	8.87	.003
rRT-PCR +, fever, cough, odynophagia, dyspnea, irritability, diarrhea, thoracic pain, chills,	1.43	1.39 to 1.47	51.77	.00001

Models	OR	CI95%	Likelihood Ratio Test	*P*-Value
headache, myalgia, arthralgia, attack on the general state, rhinorrhea, polypnea, vomit and abdominal pain				
rRT-PCR +, fever, cough, odynophagia, dyspnea, irritability, diarrhea, thoracic pain, chills, headache, myalgia, arthralgia, attack on the general state, rhinorrhea, polypnea, vomit, abdominal pain and conjunctivitis	1.43	1.40 to 1.47	11.55	.0007
rRT-PCR +, fever, cough, odynophagia, dyspnea, irritability, diarrhea, thoracic pain, chills, headache, myalgia, arthralgia, attack on the general state, rhinorrhea, polypnea, vomit, abdominal pain, conjunctivitis and cyanosis	1.43	1.40 to 1.47	1.8	.18
rRT-PCR +, fever, cough, odynophagia, dyspnea, irritability, diarrhea, thoracic pain, chills, headache, myalgia, arthralgia, attack on the general state, rhinorrhea, polypnea, vomit, abdominal pain, conjunctivitis and anosmia	1.53	1.49 to 1.57	3,498.20	.00001
rRT-PCR +, fever, cough, odynophagia, dyspnea, irritability, diarrhea, thoracic pain, chills, headache, myalgia, arthralgia, attack on the general state, rhinorrhea, polypnea, vomit, abdominal pain, conjunctivitis, anosmia and dysgeusia	1.53	1.49 to 1.57	40.02	.00001

Source: SINAVE/DGE [4]

6.1 Discussion

The sample consisted of 100,919 records obtained from the SINAVE/DGE database dated October 2, 2020. 810 records were eliminated due to not having the result of the rRT-PCR test, leaving 100,109 records of suspected cases. Of the 100,109 suspected cases as of October 2, 2020, 41.69% were positive for SARS-CoV-2 and much lower numbers for other respiratory viruses (Table 1).

Among the confirmed cases, women predominated 51.38% and the age group from 12 to 49 with 63.77% (Table 2). In a series of 99 patients in Wuhan, China, males predominated (68%) with a mean age of 55.5 ± 13.1 years [24].

Liu et al. [25] report a slight increase in affected women (52.3%) with 39.8 ± 17.1 years, 97.7% had fever, 56.8% cough, 52.3% myalgia, 40.9% headache. The frequencies of the clinical data are different from those reported in the Guanajuato population, where headache and cough prevailed, although women also predominated (Table 4).

In a series of 1,099 infected with SARS-CoV-2, reported by Guan et al. [26], 55.1% were between 15 and 49 years of age, with 58.1% predominating males and had fever 21.7%, cough 67.8%, myalgia 14.9%, and chills 11.5%. These figures are different from those reported among patients with COVID-19 residing in the state of Guanajuato since headache, cough, and fever predominated in them (Table 4).

The clinical data reported in Chinese and Mexican populations among those with COVID-19 are like many other viral respiratory tract infections, so we should not just rely on clinical data for the COVID-19 diagnosis. The diagnostic method is rRT-PCR [12].

From the operational definition of suspected cases in March 2020, which was to have two of the following symptoms: cough, fever, or headache, accompanied by at least one of the following signs or symptoms: dyspnea (severity data), arthralgia, myalgia, odynophagia / pharyngeal burning, rhinorrhea, conjunctivitis, chest pain [2], changed on August 24, 2020, in Mexico, to anyone with one

of the following: cough, fever, dyspnea (seriousness) or headache, accompanied by one of the following: myalgia, arthralgia, odynophagia, chills, chest pain [22]. The change described resulted in an increasing number of people tested by rRT-PCR.

From the signs and symptoms associated with the new coronavirus disease, none is pathognomonic for SARS-CoV-2. It is verified in Table 4 since 83.06% of the discarded cases had a headache, 75.75% cough, 56.19% odynophagia, 55.89% myalgia, 52.59% fever and 41.76% dysgeusia.

With the logistic regression models, fever showed the highest effect on being a confirmed case of COVID-19 (OR 1.72), followed by the model with fever, cough, and odynophagia (OR 1.71). Practically all the signs and symptoms improved the model, except cyanosis. In the last logistic regression model fitted, fever, cough, dyspnea, chills, myalgia, arthralgia, and general condition attack still have a statistically significant effect on being a COVID-19 confirmed case. On the other hand, odynophagia, irritability, headache, rhinorrhea, abdominal pain, conjunctivitis, anosmia, and dysgeusia had a preventive effect on having a positive rRT-PCR test result for SARS-CoV-2. Diarrhea and vomiting did not influence having positive rRT-PCR after adjusting for the rest of the clinical data.

7. CONCLUSION

Fever, cough, and odynophagia show a high effect on having a positive rRT-PCR test result.

There are no pathognomonic clinical data of COVID-19. They are like another respiratory viral infection.

Clinical data which individually shown a relationship with a positive rRT-PCR test result lost this relationship after adjusting by other clinical data.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

The protocol was approved by Bioethics Committee of Campus Celaya-Salvatierra of the University of Guanajuato in México with registry CBCCS-05230042020.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 12
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Diagnostic Accuracy and Pitfalls in Fine Needle Aspiration Cytology of Salivary Gland Lesions: An Advanced Study Approach

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ABSTRACT

Background: Fine Needle Aspiration cytology (FNAC) is an essential diagnostic method used to evaluate salivary gland lesions. However, at times, diverse morphological patterns and overlapping features between benign and malignant lesions becomes challenging and difficult to give a definitive diagnosis.

Aims/Objectives:

1. To compare the findings of preoperative FNAC with their histopathological types.
2. To discuss the causes for discordancy and identify the potential pitfalls in cytological diagnosis.

Materials and Methods: An observational analytical study was carried out over a 4-year period to review the cases of patients with salivary gland lesions who underwent FNAC in a medical college, hospital. Taking histopathological diagnosis as gold standard, the cytological diagnosis of the cases was compared and the causes of discrepancies were evaluated. Sensitivity, specificity, accuracy, positive predictive value and negative predictive value was calculated.

Results: In the present study, out of 137 cases, cyto- histological correlation was available in 46 cases. Pleomorphic adenoma was the commonest lesion in the study. The diagnostic value of FNAC was: Sensitivity 66.7%, Specificity 97.4%, Positive Predictive Value 80%, Negative Predictive Value 95% and Diagnostic Accuracy 93.3%. False positive diagnosis was rendered in Warthin's tumor whereas false negative diagnosis was given in mucoepidermoid carcinoma.

Conclusion: FNAC is useful in the preoperative diagnosis of salivary gland lesions. Pitfalls in cytologic diagnosis were due to errors in sampling, cystic lesions and interpretation of smears. Accuracy depends on experience, and this method provides superior advantages for the clinicians and the patients.

Keywords: FNAC salivary gland; diagnostic accuracy; pitfalls.

1. INTRODUCTION

Fine needle aspiration cytology (FNAC) as a diagnostic procedure is well established and commonly used in the diagnosis of salivary gland lesions, which account for less than 3% of all head and neck tumors. It is a sensitive and specific technique as compared to incisional biopsy and frozen section [1,2] besides has gained popularity due to ease of performance, coupled with the accessibility of quick stains on air-dried smears, low morbidity and rapid diagnosis of superficial and deep-seated masses. Although management of salivary gland lesions is dependent on "triple assessment" which comprises of clinical, radiological together with cytological examination, FNA plays an integral part, as clinically it is difficult to distinguish benign and malignant lesions besides lack of characteristic radiological features towards a definitive diagnosis [3].

FNA obviates surgery and is beneficial in preoperative information, guides the clinician in further management. However, majority of the salivary gland lesions have varied morphological patterns and

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overlapping features between non-neoplastic, benign and malignant lesions thus making it difficult to give an accurate diagnosis [4-8].

This study aims 1) To determine the diagnostic yield of FNAC of salivary gland lesions and compare it with histopathological diagnosis. 2) To discuss the causes for discordancy and identify the potential pitfalls in cytological diagnosis.

2. MATERIALS AND METHODS

An observational analytical study was carried out over a 4-year period to review the cases of patients with salivary gland lesions who underwent FNAC in a medical college, hospital. The study was approved by the institution ethical and research committee.

FNAC was performed using a 22 – 23-gauge needle attached to a disposable syringe under aseptic conditions after prior consent. The material was aspirated and smeared onto a clean glass slides and thin smears were prepared between two slides. The air dried and ethanol fixed smears were stained with MGG (May Grunwald's Giemsa) and Pap (Papanicolaou) stain. Forty-six (46) patients underwent excision biopsies. After gross examination of specimens, the representative sections were processed and examined by H & E (Hematoxylin and Eosin) methods. Special stains were performed when necessary. Taking histopathological diagnosis as gold standard, the cytological diagnosis of the cases was compared and the causes of discrepancies was evaluated. Sensitivity, specificity, accuracy, positive predictive value and negative predictive value was calculated.

3. RESULTS

Among the total 137 patients who had undergone FNAC, 75 (54.7%) were males and 62 (45.3%) were females (M: F ratio = 1.2:1). 98 cases (71.5%) presented with Parotid gland involvement, submandibular gland and minor salivary gland accounted for 27.7 % (38 cases) and 0.7 % (one case) respectively.

Among the 137 cases who underwent FNAC, 3 cases (2.18 %) were inadequate due to sparse cellularity, 59 cases (43. 06 %) were diagnosed as non-neoplastic, while among the 75 cases (54.74%) diagnosed as neoplastic, 65 cases (47.44%) were rendered benign while 10 cases (7.29 %) were malignant on cytology.

Cytohistopathological correlation was attainable for 46 cases (33.57%), of which one was excluded due to sparse cellularity. The relation between cytological and histological diagnosis is shown in (Table 1).

Taking histology as the "gold standard, the diagnostic value of FNAC after excluding the inadequate cases was as follows: Sensitivity for diagnosis of malignant salivary gland lesion was 66.7%, Specificity 97.4%, Positive Predictive Value 80%, Negative Predictive Value 95% and Diagnostic Accuracy 93.3%.

06 FNAC cases showed discordant diagnosis in specific typing of the lesion (Table 2). False positive diagnosis was rendered in Warthin's tumor (WT) whereas false negative diagnosis was given in mucoepidermoid carcinoma. Three cases remained benign on histology however tumor type was changed.

3.1 Summary of the Discordant Cases are as Follows

The case 01 was mucoepidermoid carcinoma with spindle cell component which was incorrectly diagnosed as myoepithelioma. The loosely cohesive spindle cells in a metachromatic fibrillary matrix was misinterpreted as myoepithelioma (Fig. 1a).

The case 02 was mucoepidermoid carcinoma incorrectly interpreted as WT. The aspirate obtained was mucoid. On review of the smears the intermediate cells with eosinophilic cytoplasm was misinterpreted as oncocytes (Fig. 1b).

Table 1. Relation between FNAC diagnosis and final histopathologic diagnosis

Cytological Diagnosis	No. of Cases	HISTOLOGIC Diagnosis	NO.Of Cases
Non-neoplastic	8		
Epidermal cyst	1	Epidermal cyst	1
Sialdenosis	1	Pleomorphic adenoma	1
Chronic sialadenitis	4	Chronic sialadenitis	4
Abscess	1	Abscess	1
Benign epithelial cyst	1	Benign epithelial cyst	1
Benign tumors	32		
Pleomorphic adenoma	21	Pleomorphic Adenoma Chronic Sialdenitis	20 1
Warthin's tumor	8	Warthin's tumor Mucoepidermoid carcinoma	7 1
Cystic lesion	1	Warthin's tumor	1
Myoepithelioma	2	Myoepithelioma	1
		Mucoepidermoid carcinoma with spindle cell component	1
Malignant tumors	5		
Mucoepidermoid carcinoma	1	Mucoepidermoid carcinoma	1
Adenoid cystic carcinoma	1	Adenoid cystic carcinoma	1
Squamous cell carcinoma	1	Warthin's tumor	1
Low grade salivary gland neoplasm with atypical cells	1	Low- grade Mucoepidermoid carcinoma	1
Squamous cell carcinoma /High grade Mucoepidermoid carcinoma	1	Metastatic squamous cell carcinoma (Parotid Lymph Node)	1

Table 2. 06 FNAC Cases of discordant diagnoses in specific typing of the lesion

Cases	Age	Sex	Site	Cytologic diagnosis	Histo pathological diagnosis
01	58	M	PAROTID	Myoepithelioma	Mucoepidermoid carcinoma with spindle cell component
02	54	F	PAROTID	Warthin's tumor.	Mucoepidermoid carcinoma
03	47	M	PAROTID	Squamous cell carcinoma	Warthin's tumor
04	53	M	PAROTID	Benign salivary gland cyst	Warthin's tumor
05	58	M	PAROTID	Sialadenosis.	Pleomorphic adenoma
06	61	M	SUBMANDIBULAR	Cellular pleomorphic adenoma	Chronic sialadenitis

The case 03 was WT incorrectly interpreted as malignant squamous neoplasm. FNA showed necrotic debris which appeared as thick, granular material with few clusters and singley dispersed epithelial (squamous) metaplastic cells with atypia, mimicking squamous cell carcinoma (Fig. 2a).

The case 04 was WT misinterpreted as benign salivary gland cyst. Aspirated 3ml of thick material. FNA showed dirty background with normal acinar cells (Fig. 2b).

The case 05 was pleomorphic adenoma which was incorrectly diagnosed as sialdenosis. The smear showed normal acinar and ductal epithelial cells (Fig. 3a).

The case 06 was chronic sialadenitis incorrectly diagnosed as cellular pleomorphic adenoma. The smear showed abundant normal acinar cells, fibrillary fibrous stroma and occasional lymphocytes (Fig. 3b).

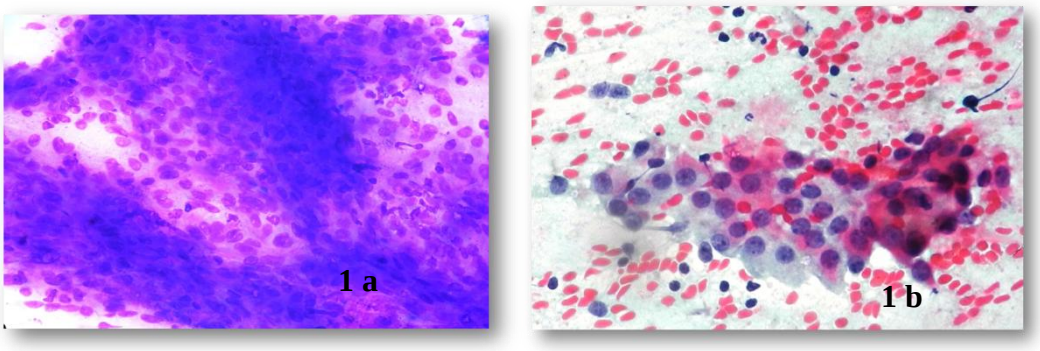

Fig. 1a. Case 1 shows FNA of mucoepidermoid carcinoma with spindle cell component misdiagnosed as myoepithelioma (Pap, 10x)
Fig. 1b. Case 2 shows FNA of mucoepidermoid carcinoma showing intermediate cells with eosinophilic cytoplasm misinterpreted as oncocytic cells of WT (Pap, 40x)

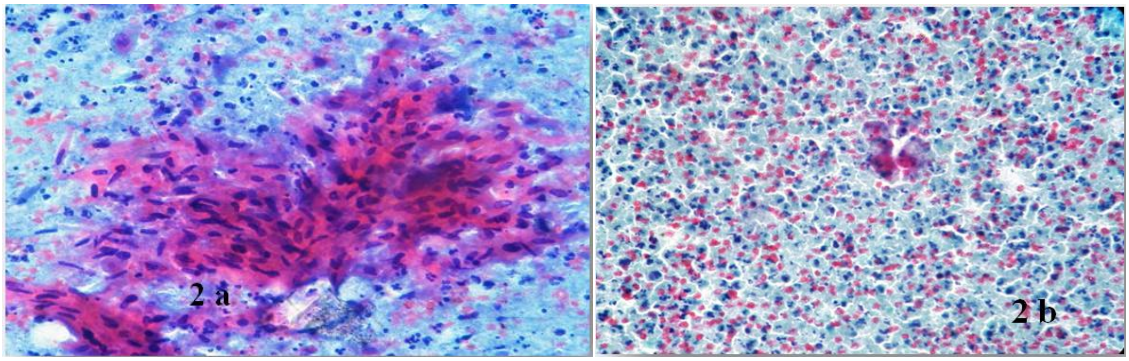

Fig. 2a. Case 3 shows FNA of WT, squamous metaplastic cells with atypia , mimicking squamous cell carcinoma (Pap, 40x)
Fig. 2b. Case 4 shows FNA of WT, incorrectly diagnosed as Benign cystic lesion (Pap stain, 10x)

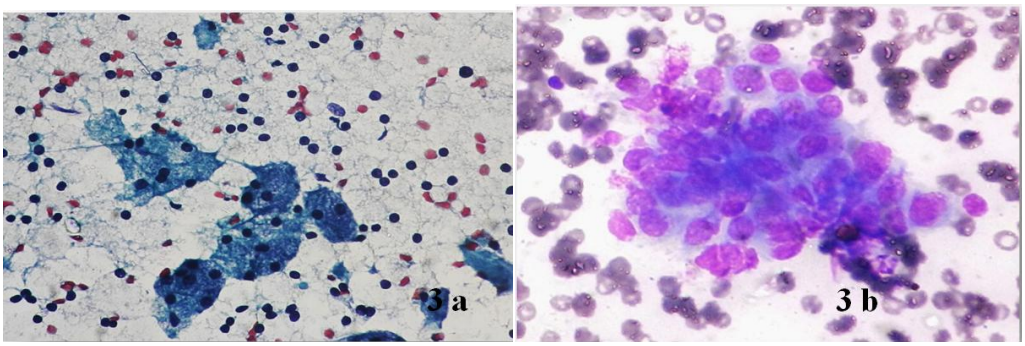

Fig. 3a. Case 5 showing a pitfall in sampling error of pleomorphic adenoma incorrectly diagnosed as siadenosis (Pap stain, 40x)
Fig. 3b. Case 6 shows Chronic sialadenitis misinterpreted as cellular Pleomorphic adenoma (MGG stain 40x)

4. DISCUSSION

FNAC of salivary gland lesions is beneficial, quick, reliable, minimally invasive and guides in assessing the whether the lesion is of salivary gland origin or a clinical mimic, subsequently helps in categorizing them into cystic, inflammatory or neoplastic process [9]. Sensitivity, specificity and accuracy of FNA diagnosis of salivary glands obtained in this study is comparable with the data obtained in similar studies done previously (Table 3).

Diagnostic problems in FNA cytology of salivary glands is debated by many authors [9]. Rare significant post-FNA tissue changes such as tumor infarction, hemorrhage, metaplasia may sometimes obscure the histological diagnosis of the resected specimens [4]. Various interpretational challenges due to heterogeneity of benign and malignant tumors and overlapping cytomorphological features account for the indeterminate or “suspicious” diagnosis [10].

False positive and false negative diagnoses were pointer towards problems and pitfalls in cytologic interpretation.

The false negative results reported in literature ranged from 0-37% [2]. In our work it was 4.44%, this was due to misdiagnosis of 2 cases, one case each of myoepithelioma and WT misdiagnosed as mucoepidermoid carcinoma. According to Orell et al. [7] a definitive diagnosis of mucoepidermoid carcinoma requires the coexistence of mucin-secreting cells and cells with squamous differentiation. The challenge is not only in relation to cytodiagnosis but also in cytological typing [11]. False-negative diagnoses usually occur due to fluid causing dilution of tumor cells, inflammatory cells and debris obscuring the tumor cells. Rarely the bland-looking intermediate cells being misinterpreted as benign cells [9,12] as observed in our case.

Table 3. Comparative analysis of sensitivity, specificity, accuracy of FNAC

Study	No of cases	Sensitivity	Specificity	Diagnositic accuracy	PPV	NPV
Present study	46	66.7%	97.4%,	93.3%.	80%	95%
Yadi Rama Raju etal10	75	83.3%	97.7%	92%	96.1%	89.8%
StramandinoliRTetal11	79	82.3%	68.2%	87.7 %	68.2%	87.7%
Iqbal M et al 12	49	62.5%	96.97%	96.4%		
Rehman H et al13	50	53.28%	88.57%	78%	72.7%	79.9%

The spindle cell component in a mucoepidermoid carcinoma is however rare as seen in our case and only a few case reports have been described in literature. On reviewing the smears, only spindle cell component was seen which led to an erroneous diagnosis of myoepithelioma. Neoplastic myoepithelium in salivary gland tumors, is prone to assume a spindle cell configuration and has been reported to undergo dedifferentiation [13].

The false positive rates reported in the literature ranged from 0-10%. In our series, it was 2.22%, due to misinterpretation of WT on FNAC as squamous cell carcinoma. WT have a characteristic cytomorphologic appearance represented by three main components —oncocytes, lymphocytes, and the fluid background. Cytological difficulties can be divided into three areas:

1. Absence of one or more diagnostic components
2. Squamoid pattern; and
3. Mucinous metaplasia. The fluid imparts a dirty background appearance that may be confused tumor necrosis [14,15,16].

Mucoepidermoid carcinoma, squamous cell carcinoma, and oncocytoma are commonly misdiagnosed as WT. In the present case, FNA showed dirty necrotic background and squamous metaplastic cell clusters with atypia. However, oncocytes and lymphocytes were not visualized in the smears which was misleading and lead to the diagnosis of squamous cell carcinoma. It has been shown that

metaplastic/reparative changes can occur in benign salivary gland neoplasms due to physical trauma induced by FNA which include squamous metaplasia, infarction, necrosis, subepithelial stromal hyalinization, acute/chronic hemorrhage, inflammation with multinucleated giant cells, granulation tissue with subsequent fibrosis, cholesterol cleft formation, pseudoxanthomatous reaction and microcystic degeneration [17]. Squamous metaplasia in our case could be attributed to previous FNA.

In one case (case 4) WT, a nonspecific diagnosis of cystic lesion was made in cytology as the smears showed mainly fluid and benign acinar cells even on repeated aspiration. On histology it turned out to be WT with predominant cystic change. Histologically, WT characteristically consists of cystic and solid areas. The cystic area is lined by layers of tall columnar oncocytic luminal cells and flattened or cuboidal basal cells, while the stroma consists of lymphocytes [11,15,18].The cytological diagnosis of cystic salivary gland lesions is rather difficult due to the wide range of lesions that enter the differential diagnosis which include chronic sialadenitis, WT, acinic cell carcinoma, pleomorphic adenoma and mucoepidermoid carcinoma [6,19].

In one case (case 5) pleomorphic adenoma was reported as sialadenosis on FNA. The smear showed only acinar and benign ductal epithelial cells. The error was mainly due to sampling which highlights the importance of multiple sampling especially in a small sized lesion.

In another case (case 6), a histologically proven chronic sialadenitis was misinterpreted as cellular pleomorphic adenoma on FNAC. This was mainly due to cellular smears and lack of acini with only ductal epithelial cells. Usually clinical correlation solves the problem. Tumor like nodules formed by focal chronic inflammation is oberved in chronic sialadenitis. Presence of epithelial cell aggregates associated with fibrillary fibrous stroma might possibly be mistaken for pleomorphic adenoma, but the fragments of ductal epithelial cells are cohesive and stroma is not chondromyxoid [7].

Our experience shows that FNA cytology of salivary gland lesions is a valuable diagnostic method. Accuracy depends on experience, and this method provides superior advantages for the clinicians and the patients.

5. CONCLUSION

FNAC is recommended as a safe and reliable technique in diagnosis of salivary gland lesions. Despite high sensitivity, pitfalls due to errors in sampling, cystic lesions and interpretation of smears lead to the misinterpretation and therefore inaccurate diagnosis Hence, a cautious approach to achieve a precise diagnoses and recognition of the limitations in cytology is recommended to avoid unwarranted surgery and planning subsequent therapeutic management.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 13
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Radionuclide Estimation of the Tumors and Compensatory Capacity of the Kidneys

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ABSTRACT

Aim: Assess the tumors and compensatory capacity of the kidneys on the basis of determining the volume of functioning kidney tissue according to single-photon emission scintigraphy (SPECT).
Materials and Methods: 65 patients with kidney parenchyma cancers, 32 patients with cysts, and 15 patients with no kidney pathology were included in our study. In addition, we looked into 57 children with a variety of non-cancerous pathologies. The ratio of the volumes of functioning tissue and particular activities between the afflicted and contralateral kidneys, as well as the ratio of the values of the obtained kidney volumes to the appropriate volume for the patient under research, based on his anthropometry data (sex and age), were employed.
Results: According to the findings, the highest compensatory renal reserve occurs when the volume of functioning tissue of the afflicted kidney decreases and the volume of the contralateral kidney increases. The cases are defined as tumours with infiltrating growth if the ratio of the affected kidney volume to the tumour and contralateral is <0.65, and the ratio of their specific activities is <0.75.
Objects are sometimes referred to as tumours without infiltrating growth.
Conclusions: As a result, the proposed integral parameters allow for the evaluation of the kidneys' compensatory potential.The value of the functional volume according to SPECT allows important information about the functioning parenchyma of the kidney in tumours to be obtained. The information used to select indications for conserving surgery.

Keywords: SPECT; functional volume; tumors and compensatory capacity of the kidneys.

1. INTRODUCTION

The most common radionuclide approaches in urology are used to assess the functional abilities of the kidneys on the basis of dynamic investigations and the identification of foci on scintigrams using planar gamma cameras or on tomoscintigraphic sections using a single-photon emission computer tomograph (SPECT). The approach of determining the volume of working organ tissue by summing the volume parts that make up the image of the organ is far less common. This study is based on a method we developed for using SPECT to determine the function of kidney tissue [1]. A practical quantitative single photon emission computed tomographic technique based on an empirical threshold analysis permits accurate measurements in humans of drug delivery and absorbed radiation dose [2-4]. Our studies have shown that with SPECT, it is possible to accurately determine the volume of functioning organ tissue and on its basis to determine integral parameters that ensure high accuracy of diagnosis. Phantom studies have shown that for organs such as the kidney with a fixed background cutoff, the error in calculating the volume does not exceed 5.1%.

The purpose of this work is to assess the tumors and compensatory capacity of the kidneys using the values of the volume of functioning kidney tissue obtained on the basis of radionuclide methods.

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2. MATERIALS AND METHODS

Radionuclide studies were performed in adult patients using SPECT. In this study, we analyzed 97 patients (65 patients had malignant kidney tumors, 32 patients had cysts, a control group of 20 patients without pathological changes in the kidneys).

In addition to radionuclide studies, all patients underwent ultrasonic, x-ray, clinical and laboratory and morphological studies. Out of 65 patients with unilateral malignant lesions, 40 cases without infiltrating growth and 25 cases with infiltrating growth of the focus were isolated.

When processing the SPECT data, the volume of functioning tissue of the right and left kidneys was calculated.

In addition, radionuclide studies of 51 children aged 1-17 years were analyzed, in which the kidney volume was determined on the basis of static polypositional scintigraphy subtracting the volume of the ellipse.

Of these, 12 patients were diagnosed with hydronephrosis, 12 megaurether, and 27 vesicoureteral reflux. Studies were performed 6 months after surgery.

To conduct radionuclide studies, the radiopharmaceutical DMSA was used in accordance with the dosage standards.

When processing radionuclide studies, the following values were determined: Vl, Cl, Vr, Cr, where Vl is the volume of the left kidney volume in cm^3, Cl is the value of the total activity of the left kidney (imp), Vr is the volume of the right kidney in cm^3, Cr is the sum activity of the right kidney (imp).

To estimate the data on the basis of the values obtained, the following integral indicators are proposed: Vaff / Vcont; (Caff / Vaff): (Ccont / Vcont); where Vaff - the volume of the affected kidney, Vcont - the volume of the contralateral kidney, Caff / Vaff - specific activity of the affected kidney, Ccont / Vcont - specific activity of the contralateral kidney.

The question of which kidney is affected was solved based on the data of a comprehensive diagnostic examination.

In addition to these parameters, we used indicators that characterize the ratios of calculated kidney volume to the proper for the patient: Vaff / Vnorm; Vcont / V norm. According to radionuclide studies of the control group, the maximum kidney volume (Vnorm) occurs in men for 25-35 years and decreases in other age ranges.

Based on this situation and using the correction factor for each patient, you can determine the value of the proper volume by the formula:

Vnorm = Vnorm *: Kk, where Kk is the correction factor, which is set according to the dependence of the weight of the kidney on sex and age [5]. Note that the ratios of the calculated volumes of the kidneys to the proper can be determined using various diagnostic methods: ultrasound, CT and others.

3. RESULTS

Table 1 presents the results of statistical processing of integrated indicators. Considering the results obtained on the proposed parameters, the following regularities can be noted.

Parameters for a group of patients with cysts do not differ significantly from the control group. In cases of tumors with no infiltrating growth, there is a slight decrease in the volume of the affected kidney in relation to the contralateral (0.88), while maintaining the level of specific activity (0.99). At the same time, the size of the affected kidney increases with respect to the proper volume (1.16) along with the

compensatory increase in the contralateral kidney. In cases of tumors with the presence of infiltrating growth, the volume of the affected kidney decreases with respect to the contralateral kidney to 0.73, together with a decrease in the ratio of specific activities to 0.71. The decrease in these indicators is due to a decrease in the functioning of volumetric elements outside the source. The loss of the volume of functioning tissue with infiltrating growth of the tumor leads to a decrease in the specific activity of the affected kidney and, as a result, an increase in these parameters for the contralateral kidney. On the basis of the parameters Vaff / Vcont and (C / Vaff): (C / Vcont), two classes of objects can be distinguished - characteristic of tumors with and without infiltrating growth. The following decisive rule is used: objects having the ratio of the volume of the affected kidney to the volume of the contralateral kidney <0.65 and the ratio of the specific activities of the affected and contralateral kidneys <0.75 belong to the class characteristic of tumors with the presence of infiltrating growth (Fig. 1). In other cases, objects belong to a class that is characteristic of tumors without infiltrating growth (Fig. 2). With this method, the reliability of the test increases with the size of the focus. The accuracy of this method for the entire range of tumor sizes (from 2.5 to 9 cm) is 83.1%, sensitivity is 75.8%, specificity is 90.1% [6].

In addition to these parameters, the indicators characterizing the ratios of the allocated volumes of the kidneys to the proper for the examined patient, according to his age and sex, were used. In the focus of the renal cell carcinoma less than 3 cm in diameter in the parenchyma of the affected and contralateral kidneys, no noticeable functional changes are detected. The volume of the kidneys within the age and sex norms. A slight loss of the volume of the parenchyma of the affected kidney is compensated for by including nephrons that make up the functional reserve of the kidneys. With a focus larger than 3 cm, an increase in the volume of functional tissue is observed. There is depletion of the functional reserve of the renal parenchyma and for the preservation of homeostasis, the processes of hypertrophy of existing nephrons are included. Both kidneys react with symmetrical compensatory hypertrophy as a single organ.

The next stage in the development of the compensatory response occurs when tumors exceed 6 cm. The reserves of the affected kidney are exhausted, the volume of its functional tissue (Vaff) begins to decrease. At this stage, most often the germination of tumor pseudocapsules and the acquisition of a focus infiltrating growth. The volume of the functional tissue of the contralateral kidney (Vcont) reaches a maximum level exceeding the average on 54% [6] due volume. The largest excess of the size of the contralateral kidney in relation to the proper volume was 72% (Fig. 1).

Therefore, the degree of compensatory response of the parenchyma of the affected and contralateral kidney and comparing them with each other, as well as with the proper volume of the kidneys of the patient being examined, can provide data on the growth pattern of the tumor site, predict the level of compensatory hypertrophy. This approach is useful for assessing the functional parameters of the parenchyma after surgical treatment (nephrectomy and resection of the kidney with a tumor) at 6 and 12 months. As a result of a repeated SPECT study in patients after nephrectomy, it is possible to compare the volume of the contralateral kidney with the data prior to surgery. There is an increase in the volume of the contralateral kidney relative to that due 6 months after surgery, an average of 69%. Further increase in the volume of the contralateral kidney (after 12 months) is not observed. As a result of resection of compensatory hypertrophy, the contralateral kidney is exposed (on average 54%).

Table 2 shows the statistical integrated indicators for different clinical groups, as well as data from patients without kidney damage in children.

Studies were performed 6 months after surgery.

Consider changes in these indicators for different clinical groups.

3.1 Hydronephrosis

Among the patients with this diagnosis, two groups can be distinguished. The main group. A definite decrease in the specific activity of the affected kidney in relation to the contralateral kidney, the

volume of the affected kidney is not reduced in relation to the proper, the volume of the contralateral kidney corresponds to the proper. The second group. A sharp decrease in the specific activity of the affected kidney in relation to the contralateral (0.11-0.12), the correspondence of the affected volume to the due (0.94 - 0.98) and an increase in the volume of the contralateral kidney in relation to the proper. (1.36 - 1.5), which is explained by a high level of compensatory inclusion of reserve nephrons. MEGAURETER. In the majority of patients in this group, there is a significant decrease in the volume of the affected kidney in relation to the proper. In a number of patients, this decrease is accompanied by a significant decrease in the ratio of the specific activities of the affected and contralateral kidneys and a sharp increase in the volume of the contralateral kidney relative to the proper (up to 1.7).

Table 1. Statistical indices for various clinical groups with SPECT kidney

	Vaff/Vnorm	Vcont/Vnorm	Vaff/Vcont	(C/Vaff)/(C/V)cont
Norm			1.0±0.02	0.96±0.02
Cyst	1.06±004	1.08±004	1.0±0.07	0.98±004
Tumor without inf. growth	1.16±0.07	1.22±0.05	0.88±0.05	0.99±0.06
Tumor with inf. growth	0.93±0.09	1.54±0.06	0.73±0.08	0.71±007

Table 2. Statistical indices for various clinical groups for radionuclide studies of kidneys in children

	Vaff/Vnorm	Vконт/Vnorm	Vaff/Vcont	(C/Vaff)/(C/V)cont
Norm			1.0±0.02	0.96±0.02
Hydronephrosis	1.07±0.12	1.22±0.07	1.0±0.15	0.65±0.13
Megaureter	0.53±0.11	1.21±0.2	0.51±0.14	0.86±0.08
VUR	0.64±0.13	1.05±0.09	0.71±0.14	0.88±0.03

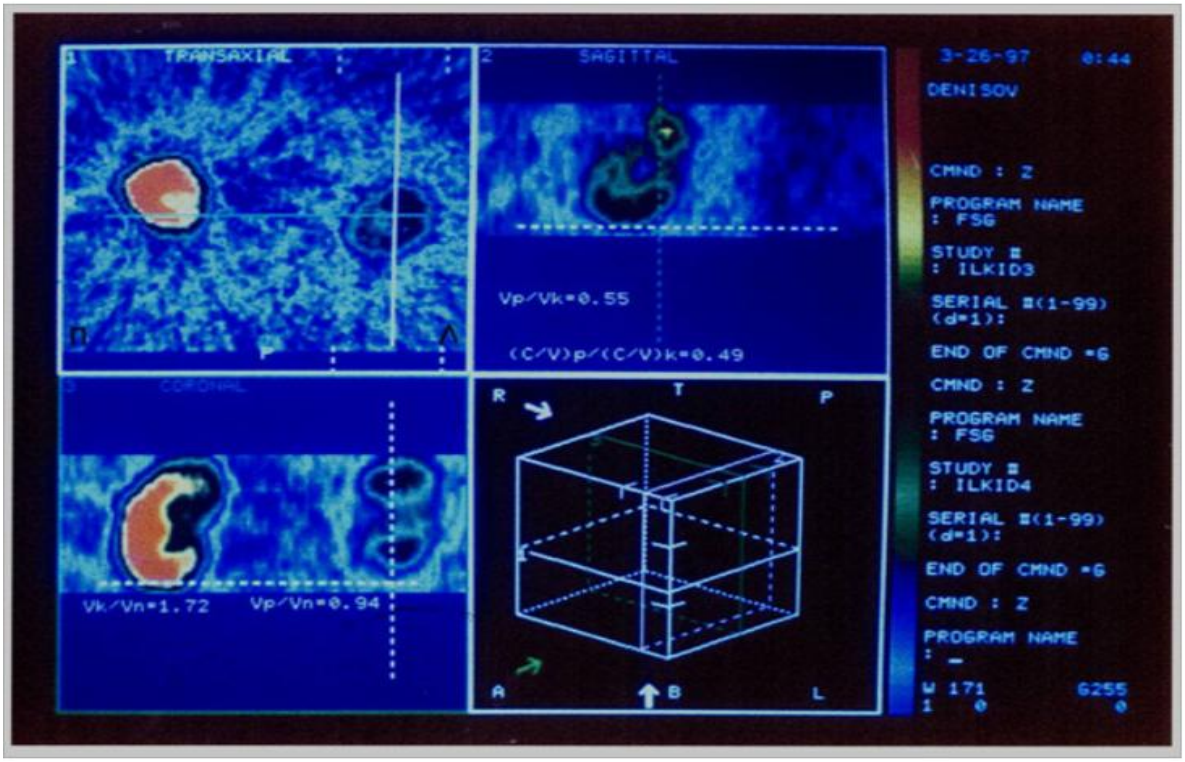

Fig. 1. SPECT in case of left kidney cancer with infiltrating growth
Vaff / Vcont = 0.55; (C / V) aff / (C / Vcont) = 0.49; Vkont / VNorm = 1.72

3.2 Vesicoureterall Reflux

The most pronounced change is a decrease in the volume of the affected kidney with insignificant changes in the ratio of specific activities and the size of the contralateral kidney.

Thus, the proposed indicators are sensitive for detecting the extent of the lesion and evaluating the compensatory possibilities for radionuclide studies of kidneys in children.

In Fig. 3 shows an example of an 11 year old patient with hydronephrosis of the left kidney. Integral indicators have changes with respect to corresponding to the data for the group norm, which indicates a stable phase of the compensatory process. Fig. 4 shows an example of a 17 year old patient who also has hydronephrosis of the left kidney. The values of the integral indicators are significantly changed in relation to the data of the group norm, which indicates the maximum possibilities of the compensatory process.

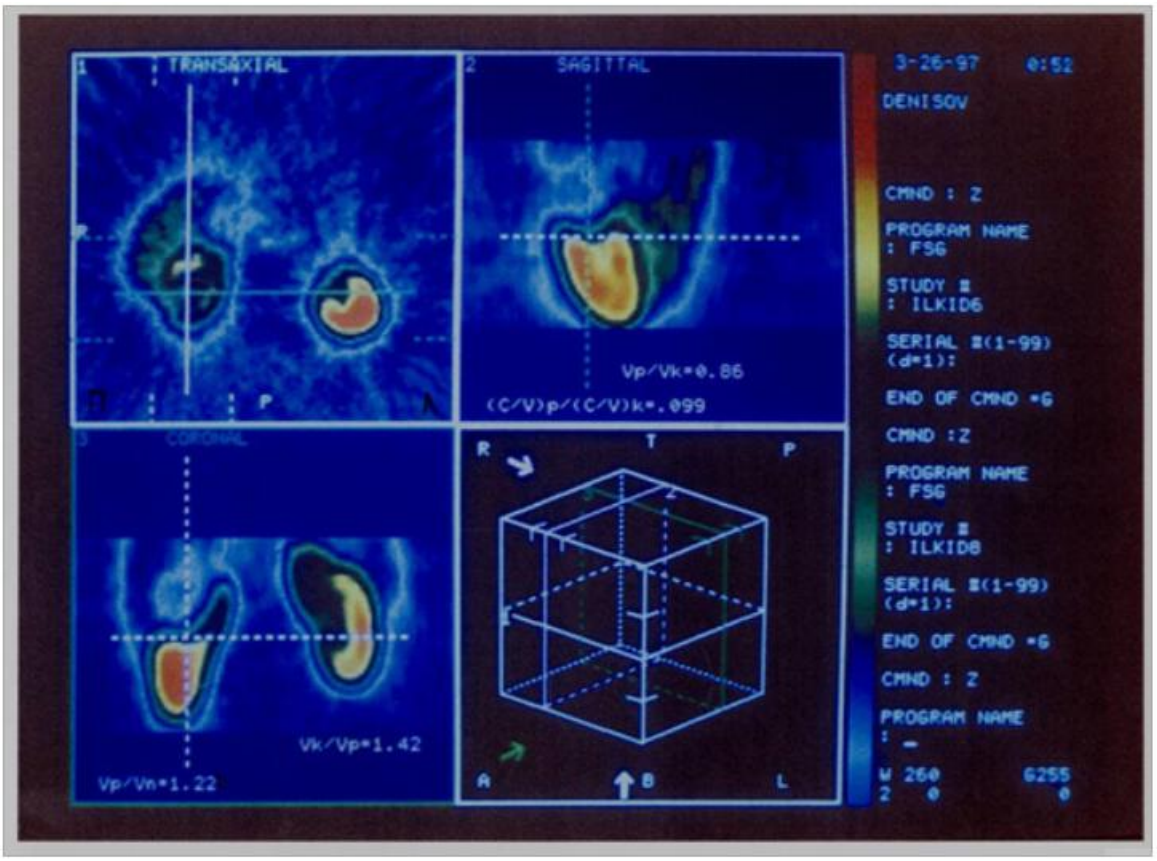

Fig. 2.SPECT with cancer in the upper segment of the right kidney with no infiltrating growth
Vaff / Vcont = 0.85; (C / V) aff / (C / Vcont) = 0.99; Vkont / VNorm = 1.22

Summarizing the results obtained for clinical groups, the following stages of compensatory processes in the kidneys can be distinguished.

1. Change in the volume of the affected kidney in relation to the proper with minor changes in other parameters.
2. Reduction of the ratio of specific activities of the affected and contralateral kidney with minor changes in other parameters.
3. Significant decrease in the ratio of specifiac activities of the affected and contralateral kidneys with a sharp increase in the volume of the contralateral kidney in relation to the proper (up to 1.72) and a decrease in the volume of the affected kidney.

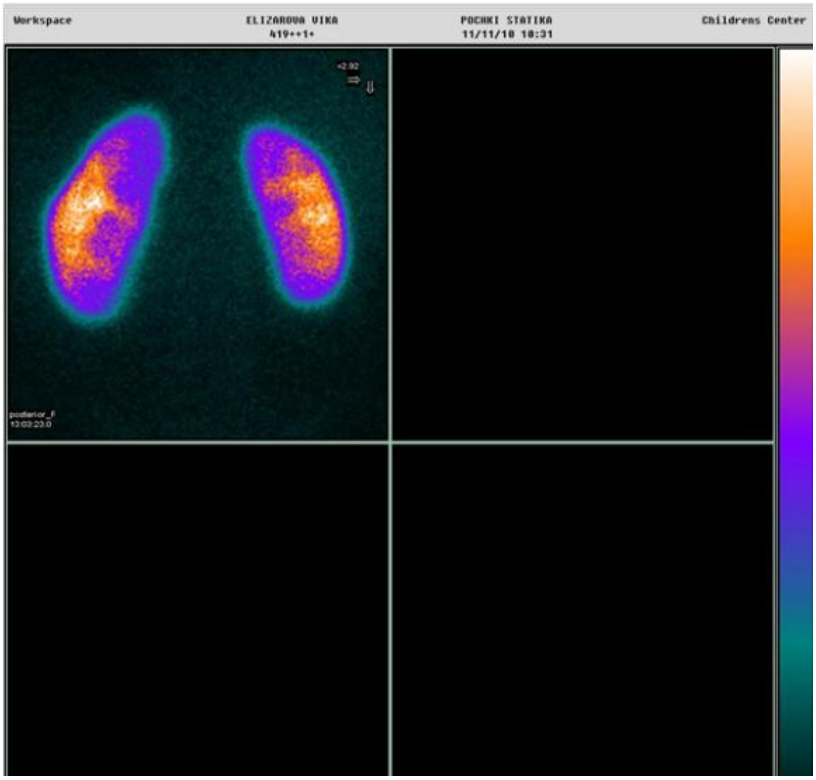

Fig. 3. Multiplanar kidney study of an 11-year-old female patient with hydronephrosis of the left kidney

Vdam / Vcont = 1.38, (C / Vdam / (C / Vcont) = 1.14. Vcont / Vnorm = 1.24

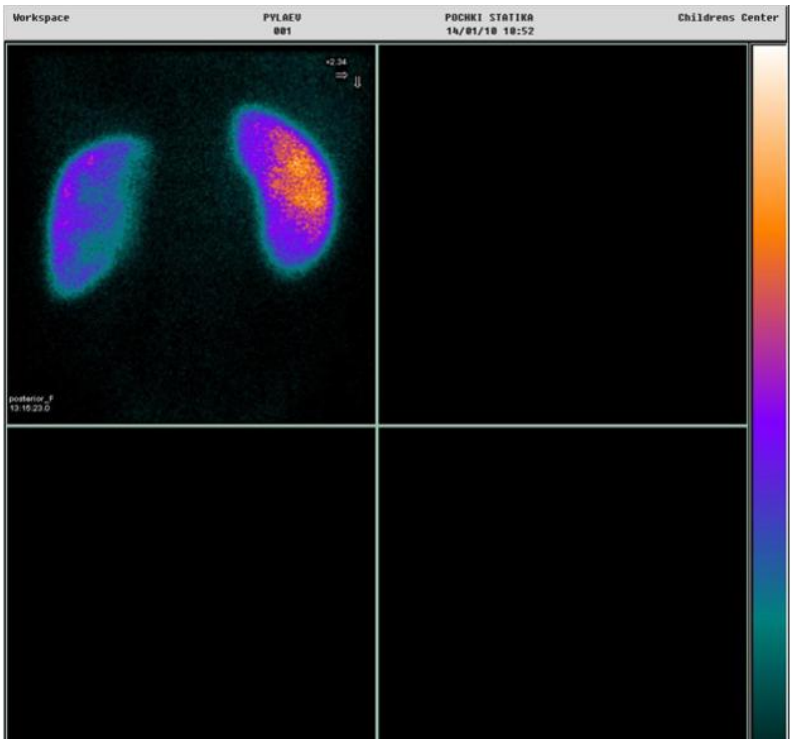

Fig. 4. Multiplanar kidney study of a 17-year-old patient with hydronephrosis of the left kidney

Vdam / Vcont = 0.86, (C / V) dam / (C / Vcont) = 0.66, Vcont / Vnorm = 1.47

4. SUMMARY

Thus, conducted studies of adult and pediatric patients with various kidney lesions showed that using parameters based on determining the volume of functioning kidney tissue it is possible to evaluate tumors and the compensatory possibilities of the parenchyma of the affected and contralateral kidneys with the help of SPECT or planar radionuclide studies. Identification of the peculiarities of the changes in the anatomical and functional state of the parenchyma allows us to approach objectively the choice of tactics of surgical intervention and treatment, and dynamic monitoring of urological patients.

Results estimation of tumors are based on the revealed dependence: even a small malignant tumors leads to decreased radiopharmaceutical accumulation in the tissues surrounding the node, which results in decreased functioning tissue of entire organ. With the malignant node invasion this pattern is even more notable.

5. CONCLUSION

Radionuclide Estimation of the kidneys allows to obtain important information about state functioning parenchyma at tumors and compensatory capacity of kidneys. The results are based on the revealed dependence: even a small malignant tumor leads to decreased radiopharmaceutical accumulation in the tissues surrounding the node, which results in decreased functioning tissue of entire organ.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 14
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Is Silver Diamine Fluoride Really a Magic Alternative in Pediatric Caries Management? : An Advanced Clinical Approach

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ABSTRACT

Aim and Objective: To review the success of silver diamine fluoride (SDF) in the management of caries in children.
Background: The current caries management strategy is based on a biological model. Children with early childhood caries require behaviour management as well as effective and thorough caries management, for which an algorithm-based approach is proposed.Silver diamine fluoride functions as a tertiary preventive procedure, reducing the negative consequences of established disease (cavity) and restoring, thus improving the child's quality of life. It's a magic bullet that combines silver's antibacterial properties with highly concentrated fluoride's remineralizing properties.
Review Results: Previous systematic reviews found that SDF was effective in halting caries in primary teeth and root caries in adults. Because caries removal is not required, SDF application is appropriate when other caries management modalities are unavailable or impractical. Aside from the arrested lesion turning black, no significant complication of SDF use in children was reported.
Conclusion: Despite the unappealing black staining, a significant number of parents chose SDF over advanced pharmacological behaviour management techniques, and it is truly the magical alternative in children as it supports the contemporary "biological approach" to caries management.
Clinical Significance: During the COVID-19 pandemic, SDF allows the interruption of aerosol-generating procedures and serves as an interim treatment procedure to arrest dentin caries and prevent the development of pulpitis, which requires further intervention with an aerotor. Most importantly, theSDF application is child-friendly and provides exceptional success rates when used where indicated.

Keywords: Caries management; dental caries; pediatric dentistry; silver diamine fluoride; silver fluoride bullet.

1. BACKGROUND

Caries of the teeth is a chronic condition that affects youngsters all over the world. Even though caries indicators have decreased in recent years, dental caries is heavily prevalent in the lower socioeconomic groups, similar to diseases related with community disparity [1]. “Early childhood caries (ECC), the presence of 1 or more decayed, missing, or filled tooth surfaces (dmfs) in any primary tooth in a preschool-aged child”, has been on the rise in many countries and is recognized as an important public health concern by the American Dental Association [2].

If ECC is left untreated, it can affect a child's growth, body weight, oral health-related quality of life, and school attendance and performance [3,4]. Because children with dental caries frequently have many carious lesions, managing this disease is difficult. Almeida et al. found that children with ECC who were given general anaesthesia had a higher risk of developing caries in their permanent teeth [5]. Because adult dental disease has its origins in childhood, effective caries prevention for children is

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essential. Dental caries continues to be a severe oral health problem despite a decrease in its prevalence over the past few decades. The contemporary philosophy of caries management has shifted from the traditional approach to a newer medical one, that frequently includes utilization of fluoridated and antimicrobial agents [6,7,8].

Caries risk assessment along with dietary advice, toothbrushing (toothpaste) advice, fissure sealants, and fluoride varnish are the few principal evidence-based preventive interventions for a child that is delivered by the oral healthcare team [9–11]. Previous literature shows that when the salivary components are inadequate and the bacterial challenge is high, the caries prevention that occurs as a result of remineralization (naturally or as a result of fluoride agents) suffers. Therefore, there is a pressing need to find alternatives for biofilm modification and to intensify the remineralization process which will further help in minimizing the caries experience, thus uplifting overall oral health [12]. Whenever dental caries prevention fails, a child is subjected to an increased risk of pain and infection. Therefore, the disease must be addressed without delay to manage this risk. Thus, this article aims to review the current concepts for caries management and whether silver diamine fluoride (SDF) can be used as a successful alternate caries management agent in children.

Understanding Caries Management: Current Concepts In 2018, Meyer et al. proposed an algorithm that can be referred to by clinicians when selecting disease management strategy and a behavior for early childhood caries [13]. This algorithm guides dental health professionals while providing counsel to caregivers about benefits, risks, and alternative options [13]. Advanced pharmacological behavior guidance techniques like general anesthesia and sedation are often needed to carry out restorative procedures, requiring tooth preparation in children including children with special healthcare needs. These paths have additional health risks and drawbacks (e.g., mortality risks, neurological deficits in young children), and often are inaccessible due to increased costs [14]. Moreover, “repeated or lengthy use of general anesthetic and sedation drugs during surgeries or procedures in children younger than three may affect the development of children’s brains” [15].

For such children whose caregivers are reluctant to pursue pharmacological management alternatives and those who cannot be sedated safely, non-surgical management strategies may provide reasonable alternatives to traditional restorative care. Children who are uncooperative for treatment due to age or intellectual disability as well as those who are obese or medically compromised and may be at increased risk of a compromised airway require minimal intervention approaches for non-surgical caries management.

The current guidelines on “Use of Vital Pulp Therapies in Primary Teeth with Deep Caries Lesions” published by the “American Academy of Paediatric Dentistry” in 2017 recommended that no vital pulp therapy procedure; namely, indirect pulp therapy, direct pulp capping, or pulpotomy is superior to the other. The systematic review and meta-analysis showed that success after 24 months with indirect pulp therapy was 94.4%, with direct pulp capping was 88.8%, and after pulpotomy was 82.6%. Moreover, the success of indirect pulp therapy was independent of the medicament used [16]. Therefore, the choice of vital pulp therapy in primary teeth with deep carious lesions should be based on a biological approach.

According to a recently updated Cochrane systematic review for the management of dental caries in primary and permanent dentition (which included eight trials in the analyses), biologically orientated techniques (partial, stepwise, and no-caries removal) when compared with complete caries removal concluded that the biological approach had greater clinical benefits over entire caries removal in the management of asymptomatic vital teeth. No significant differences were found in the longevity of the restorations. There was no significant difference even in the numbers of patients experiencing pulpal pain or infection post biological approach or complete caries removal. Additionally, there were significantly reduced pulp exposures [17]. The use of SDF is one such biological approach for caries management among others such as Hall crowns.

2. SILVER DIAMINE FLUORIDE

Interest in the use of silver diamine fluoride (SDF) has been rapidly increasing. Even though SDF had been used off-label for caries arrest; was recently approved (code D1354) as an interim caries

arresting medicament [18]. The first product to become commercially introduced in the United States in 2015 was advantage arrest containing 38% SDF. Systematic reviews conclude that SDF meets the "US Institute of Medicine's 6 quality aims" which are safety, effectiveness, efficiency, ease of application, patient-centered, and equitable [19].

Silver diamine fluoride, a transparent colorless liquid, is a combination of the remineralizing effects of fluoride and the antibacterial effects of silver. Thirty-eight percent SDF contains silver particles (280,000 ppm) and 38% (44,800 ppm) fluoride ion, i.e., 5% fluoride, 25% silver, 8% ammonia, and 62% water at pH 10.16 The tooth structure which is under attack by the acidic bacterial metabolic products is reinforced by the fluoride component. Moreover, SDF as a whole may also interfere and modify the biofilm causing bacterial cell death. This results in an imbalance in a local environmental niche that promotes demineralization of dental tissues [20]. Therefore, SDF becomes a unique modality available to address the carious process by altering the bacterial actions along with promoting remineralization (Tables 1 and 2). There is increased deposition of silver and fluoride in demineralized than non-demineralized dentin. Respectively, treated demineralized dentin has a higher resistance to caries bacteria than treated sound dentin. Wakshlak et al. concluded that "when bacteria killed by silver ions are added to living bacteria, the silver is re-activated, so that effectively the dead bacteria kill the living bacteria in a zombie effect" [21].

Silver diamine fluoride is indicated for interim treatment (non- surgical caries management) for patients who cannot receive traditional restorative due to pre-cooperative behavior because of age, cognitive, or physical disabilities; patients at high caries risk having many carious lesions that cannot be addressed in a single visit, unaffordability, and inaccessibility resulting from low socioeconomic status and poor awareness.

Silver diamine fluoride is indicated for cavitated caries on crown or root surfaces which are cleansable, asymptomatic, and radiographic verification [19]. It is also indicated for caries prevention in high-risk groups. It is contraindicated in people with silver allergy and stomatitis or ulcerative gingival conditions.

The growing interest in SDF revolves around its five presumed attributes: ease and simplicity of use (paint on), control of pain and infection, minimal personnel requirement, affordability of material, time and training (one minute, once per year), and its non-invasive nature [22]. Thus, SDF has the unique ability to be a magic bullet of silver-fluoride, which can both halt the cariogenic process and prevent caries.

3. CURRENT EVIDENCE ON EFFICACY OF "THE MAGIC BULLET": SDF

3.1 Caries Arrest on Deciduous Teeth

A Systematic Review and Meta-analysis was conducted by Gao et al. of which eight studies that used 38% SDF to arrest dentin caries in primary teeth were included for meta-analysis (Zhi et al., 2012; Yee et al., 2009; Llodra et al., 2005; Chu et al., 2002; Yang et al., 2002; Fukumoto et al., 1997; Ye, 1995; Wang, 1984). "After SDF treatment the mean percentage of dental caries that got arrested was 81% (95% CI, 68% to 89%; $p < 0.001$)". Several studies used SDF percentages other than 38% such as 30, 12, and 10% but it was concluded that SDF with higher concentration (38%) demonstrated a statistically significant caries-arresting effect in children. These studies did not report significant complication despite the high fluoride concentration (44,800 ppm in 38% SDF) [23]. Fung et al. conducted a study including children between 3 and 4 years where SDF 12 and 38% was applied annually and semi- annually. It was found that the caries arrest was improved with the higher concentration of SDF and that the caries arrest improved by 15% on the increasing frequency of application from annual to semi-annual. The caries arrest rates of were significantly different among the four groups namely upper anterior (77%), lower anterior (93%), upper posterior (42%), and lower posterior teeth (53%) (χ^2 test, $p < 0.001$). Fung et al. also reported that "Children with a higher Visible plaque index score had a reduced chance to have their caries arrested with once a year SDF application" [24]. However, the caries arrest rates could be improved by increasing the frequency of SDF application for such children [23]. Fung et al.'s trial also concluded that large occlusal caries

lesions in posterior teeth covered with visible plaque had a comparatively lower chance to become arrested ($p < 0.001$) [24].

Previous literature also reported that SDF was superior to glass ionomer cement or fluoride varnish in its caries arrest potential in primary teeth [23,25]. Chu et al. also suggested that removal of caries was not a prerequisite before SDF application [23]. Chibinski et al. reported that at 12 months, the caries arrest as a result of SDF application was 66% higher than any other product with active ingredients and it was 76% higher than caries remaining untreated [26].

Under a comprehensive caries management program, the AAPD recommends the use of 38% SDF as it is efficacious in caries arrest [27]. Arrest rates ranges from 60 to 91% depending on tooth surface, tooth location, presence of plaque, and application frequency. Unfortunately, it is not an option for treating pulpally involved teeth.

Therefore, it can be concluded that SDF of a higher concentration (38%) was significantly efficacious in arresting caries in the primary teeth. Bi-annual application improved caries arrest than annual application. The caries arrest efficacy of SDF is inversely related to the presence of visible plaque. The caries arrest rate was found to vary with the tooth type and region; maximum in the lower anterior region and minimum in the upper posterior region.

3.2 Caries Arrest on Permanent Teeth in Children

A review article by Rosenblatt et al. based on a single clinical trial by Llodra et al. in 2006 ($n = 373$) that comments on caries arrest after SDF in the permanent dentition. It concluded that around 77% of SDF-treated active caries became inactive in both deciduous and permanent molars [24]. Another trial with a small sample size of [25] children concluded that SDF had a better success rate than toothbrushing or glass ionomer restorations at 3 and 6 months. However, there was no significant difference in controlling non- cavitated lesions at 30 months [28].

The presence of caries arrest activity of SDF in cavitated permanent molars in children can be concluded from previous literature; however, its efficacy in controlling non-cavitated lesions is questionable and needs more evidence.

3.3 Caries Prevention in Children

Rosenblatt et al. conducted a review that included two trials to evaluate SDF's caries prevention potential. In conclusion, a preventive percentage of 70.3% (>70% on primary teeth and >60% on permanent teeth) was obtained. In the trial conducted by Llodra et al., primary molars as well as permanent molars were included. They concluded that in a span of 36 months, development of new caries in permanent teeth was significantly reduced in the SDF group (0.4 new lesions) than water control group (1.1 new lesions). Similarly, in deciduous teeth, there were 0.3 new lesions in the SDF group, whereas the water control group had 1.4 new lesions [22]. Chu et al. conducted a trial in preschool children including only the maxillary anterior teeth and found that in the SDF group, the average number of new lesions at 30 months was 0.47. However, with 4 yearly applications of fluoride varnish there were 0.7 new lesions and 1.58 new lesions in the control group (water) [25].

Therefore, from previous evidence, it can be concluded that SDF has a considerable preventive activity that is observable both in primary and permanent teeth in children. It has been shown to provide better prevention than fluoride varnish in one of the studies. More clinical trials comparing the preventive activity of SDF with other preventive agents are required to provide generalizable results.

3.4 Caries Prevention on Permanent Teeth

Liu et al. conducted a trial and concluded that the sites of pit/fissures having dentinal caries on treatment with SDF, FV, and sealant did not show a significant difference at 24 months [29]. Monse et al. found that the effectivity of one-time SDF application was less as compared to atraumatic treatment restorations after 18 months [30].

Table 1. Mechanism of action of silver as a component of silver diamine fluoride

Antibacterial activity	***Effect on dentin minerals and dentin collagen***
• Mark and Barillo 2014 Interaction occurs between silver and enzymes that block the ETS in bacteria.	• Seto and Coworkers 2017 Hardening after SDF applica- tion is attributed to its reaction with silver and not with classic fluoride-mediated remineraliza- tion.
• Lansdown 2002 When silver ions bind to the amino acids inside the bacterial cell, an organometallic complex is formed which breaks down releasing silver ions inside the cell which may cause bacterial DNA and RNA inactivation and cell membrane damage leading to the death of the bacterial cell.	• Mei, Ito et al. 2013; Mei et al. 2017 Silver chloride is the principal precipitate after SDF application.
• Russel and Hugo 1994 Interaction between silver and thiol group present in enzymes deactivates enzymes resulting in bacterial cell death.	• Mei, Ito, Cao, Lo et al. 2014 Clinically visible coal-black color indicates the presence of ar- rested caries.
• Russell and Hugo 1994 Silver ions result in DNA mutation eventually leading to bacte- rial cell death.	• Tjaderhane et al. 2013 Silver indirectly protects dentin collagen by inhibiting dentin collagenase. In comparison to silver nitrate and sodium fluoride solutions, silver from SDF was attributed to being a moderate inhibitor of MMP-8 and MMP-9 and a stronger inhibitor of cath- epsins B and K.
• Slawson et al. 1990 Silver ions can form an electro- static bond with bacterial cells, resulting in inhibition of organ- ism movement or resulting in membrane leakage or rupture.	

Table 2. Mechanism of action of fluoride as a component of silver diamine fluoride

Antibacterial activity	**Effect on dentin minerals**	**Effect on dentin collagen**
• Fluoride causes inhibition of acid production in the dental plaque.	• Fluoride may react with apatite in multiple ways. • Ion exchange between fluorides and hydroxyl ions • Fluorapatite crystal growth from • supersaturated solutions • Dissolution of apatite and formation • of calcium fluoride. (Ogard et al. 1994)	• In an *ex vivo* study with SDF treated primary teeth (exfoliated), a relatively smooth dentin surface with exposure of a few dentinal collagen fibers was appreciated in the arrested carious lesions. (Mei, Ito, Cao, Lo et al. 2014)
Fluoride in the form of hydrogen fluoride can directly inhibit cellular enzymes and enhance the membrane permeability toward protons. This is the mechanism by	Previous research found that the fluoride content in the apatite increased when the SDF con- centration increased. (Mei et al. 2017)	By comparing SDF with silver nitrate and sodium fluoride solutions, it was suggested that fluo- ride strongly inhibits MMP-2, MMP-8, and

Antibacterial activity	Effect on dentin minerals	Effect on dentin collagen
which it inhibits plaque metabolism. (Koo 2008)	Thus, it was conclud- ed that the reaction of SDF with calcium and phosphate results in the formation of fluorohydroxyapatite.	MMP-9. (Mei et al. 2012)

It can be concluded that if pits and fissures on permanent teeth have progressed to dentinal caries, SDF, fluoride varnish, and sealants demonstrate a comparable outcome after 2 years. Caries preventive activity of SDF on permanent teeth in adults requires more investigations in the form of clinical trials.

3.5 Caries Arrest and Prevention in the Elderly

In 2017, Hendre et al. in their systematic review on the use of SDF in adults included three studies on prevention and arrest of root caries. They concluded that in a study of 24 and 36 months, the preventive fraction obtained for SDF was 24 and 71%, respectively. In the third study which was conducted for a span of 30 months, SDF had a 100% greater fraction for prevention than placebo for the progression of caries. Therefore, the use of SDF is recommended for elderly who are at a greater risk for root caries and for management of dentin sensitivity [19].

4. TOXICITY AND SIDE EFFECTS

The clinical trials reported till now suggest that over 4,000 children have been treated without any major adverse events [27]. There were no cases of acute toxicity. Toxicology studies have only been done on adults using the equivalent of 1/5 of a drop. Drops are 30% larger than originally reported. A drop of SDF measuring 32.5 μL contains 1.64 to 1.76 mg of fluoride and 8.08 to 8.71 mg of silver.

Previous literature does not report any acute adverse effects after SDF application in either children or adults. Metallic taste and transient irritation of the gingiva are the minor side effects that have been reported in few participants. Only one study published on adults aimed at studying gingival erythema following SDF application 24 hours and a week after concluded that in a span of 24 hours there were a few participants who developed mild erythema of gingiva which healed itself within a week [31]. A recent clinical trial was done on young children suggested that 1 week after application, the prevalence of toothache and gingival pain was 6.6%, gum swelling was 2.8%, and gum bleaching was 4.7% as reported by the parents [19].

The main disadvantage following SDF application is the darkening of the carious tooth, which becomes difficult for parental acceptance. A study conducted in Hongkong compared parental satisfaction regarding their child's tooth appearance through a self-rated questionnaire after 30 months of 12 and 38% SDF which was applied semi-annually and annually for both formulations. It was concluded that the concentration and frequency of application did not significantly affect parental satisfaction. The overall parental satisfaction ranged from 71 to 62% [32]. A comparative survey in the United States reported that parents were more acceptable of staining on posterior teeth as compared to anterior teeth. Despite the unappealing anterior staining, there were parents who would significantly choose SDF for management of caries in their child over dental rehabilitation under general anesthesia. Most studies strongly recommend an informed consent so that the parents are well informed about the benefits and shortcomings of this treatment modality [19].

Potassium iodide application to control or reverse the staining after SDF is painted on the tooth has been studied by many investigators. Some commercial products have combined both components; SDF and KI (Riva Star, SDI, Baywater, Victoria, Australia). However, it has been reported that in adults, potassium iodide application was ineffective in reversing the staining on root surfaces, more so in the long term [33].

The undesirable effects of SDF are not only outweighed by its desirable properties in most cases but a significant number of parents are willing to compromise esthetics to avoid more invasive/risky scenarios for the delivery of treatment. Moreover, no toxicity or adverse events associated with its use have been reported till date.

5. CLINICAL APPLICATIONS

Clinicians may make a decision regarding the frequency of SDF application based on patient needs, fluoride exposure, individual caries risk factors and consider individual social determinants of health and this judgment should apply to both young and adult patients.

Before application of SDF, lip balm or petroleum jelly is applied on the lips then tooth isolation is achieved using cotton rolls. The cavitated lesion is cleaned and air-dried after which SDF is applied over the affected area for durations ranging from 10 to 3 minutes and allowed to dry. Post application rinsing is not mandatory [19].

The World Health Organization's report on "Public Health Interventions against Early Childhood Caries" in 2016, recommended that SDF is efficient in arresting dentinal caries in deciduous teeth and preventing recurrence following treatment [15]. "It recommends its use as an alternative procedure for tertiary prevention to reduce the negative impact of established disease (cavity) by restoring function and reducing disease-related complications and to improve the quality of life for children with early childhood caries".

6. CONCLUSION

Silver diamine fluoride is really the magic alternative for pediatric caries management. It supports the conservative and contemporary "biological approach" requiring no caries removal with caries lesion arrest rates being >70%. It is child friendly as it requires no anesthesia and no drills, thereby reducing the chair side time.

7. CLINICAL SIGNIFICANCE

In such times of the COVID-19 pandemic, interruption of aerosol- generating procedures is a step that is unavoidable for the safety of dental health professionals and the patients. Even though the presence of asymptomatic dental caries is not an emergent procedure, leaving it untreated will cause the tooth to continue to undergo destruction, resulting in pulpal involvement which may in turn require treatment involving aerosol-generating procedures. Therefore, SDF can be used to break this vicious cycle and help as an interim treatment procedure to arrest dentin caries. However, it must strictly not be used where it is not indicated.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 15
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Study on Emotional Intelligence and Stress Tolerance of Diabetic Physical Exercising and Diabetic Nonphysical Exercising Peoples on Critics

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ABSTRACT

Aim: Objective of the study is to enlighten the need and importance of physical exercise in developing psychological wellbeing in diabetic peoples.
Introduction :
Physical exercise is the determine way to upsurge stress tolerance and assistances to uphold a sense of emotional wellbeing.
Place and Duration of Study: Chidambaram, Cuddalore district, Tamilnadu, India. 1 year.
Methodology: For this study a normative survive method was used to collect data from diabetic physical exercising and diabetic nonphysical exercising people from Chidambaram, Cuddalore district, Tamilnadu, India. This investigate is an attempt to analyse and interpret diabetic physical exercising and diabetic nonphysical exercising people on the psychological aspect of EI. The problem of this study is a comparison of on EI of diabetic physical exercising and diabetic nonphysical exercising peoples. The sample in the present study was limited to 60 nondiabetic and 60 diabetic people.
Results: The result shows that the 'r' value obtained from the variable emotional stability and self-development on the sample of 60 on stress tolerance of diabetic physical exercising group was identified as 0.34 and 0.35 which was significant at 0.01 this shows that here remained a positive relationship between stress tolerance with emotional stability, Stress tolerance with self- development. When emotional stability increases stress tolerance increases when self- development develops stress tolerance increases. The diabetic non-physical exercising and diabetic physical exercising group significantly differ in their stress tolerance, comparatively, the mean value of 73.81 for diabetic physical exercising people with the mean value of 71.79 diabetic nonphysical exercising people is less. Hence it is proved that diabetic physical exercising people have more stress tolerance than diabetic nonphysical exercising people. This indicates that comparatively diabetic physical exercising people can withstand when deprived and tolerate critics of others because diabetic physical exercising people are more stress-tolerant when compared to diabetic nonphysical exercising people. The result of the diabetic physical exercising group's 'r' value obtained from the variable EI on the sample of 60 on stress tolerant was identified as 0.25 which was significant at 0.05.
Conclusion: There was a positive relationship between stress tolerance with EI which specifies that when EI increases stress tolerance increases for diabetic physical exercising person's vice versa.

Keywords: Emotional intelligence; stress tolerance; diabetic physical exercising and diabetic nonphysical exercising peoples.

ABBREVIATION

EI: Emotional Intelligence.

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1. INTRODUCTION

Diabetic Mellitus is caused due to endocrine disorder which leads to hyperglycemia because of insulin deficit, which later leads to an accumulation of glucose in the blood. Two major types of diabetics are Type1 and type2 diabetics. Type1 is an autoimmune disease and Type2 is noninsulin depended Mellitus. Indians are prone to develop at the early age of 10 to 15 years; cause of diabetes is due to physical inactiveness, sedentary lifestyle and lack of physical exercise. polyuria, polydipsia, polyphagia, weight loss and asthenia are the indication of diabetics. Psychological association with diabetics' lot of social, psychological study has been carried on how to manage with diabetics and how to recover them from diabetics; the concept of mental wellbeing is an integral part of diabetic management. Compare with general population depression is double the time more, patients treated with insulin are less aware of hypo- glycaemic symptom are at a risk of developing an extreme fear of hypos, chartered by disproportionate anxiety with avoiding behaviours, following negative effect on the glycemic control. Obesity and a sedentary lifestyle are common challenges among individuals at risk of diabetic foot ulcers. While substantial research exists on physical activity interventions in adults with diabetes, those at greatest risk for foot ulceration were often excluded or not well represented [1]. Type I diabetic patients, the ability of the endogenous opioid system to respond to exercise-induced stress is impaired under hyperglycemic and even under normoglycemic conditions. Considering the effect of endogenous opioids on stress tolerance, such changes may compromise exercise performance in diabetic patients [2]. The capability of individuals to identify his particular emotions and those of others is defined as EI , distinct among diverse moods and tag all properly, custom the emotional information to director intellectual and behaviour manage and to adjust feelings to acclimatize to the surroundings or accomplish individual goals [3,4]. EI defined the aptitude, capacity or skill or in event of the trait EI model, a self-perceived ability to find, assesses and manage the emotion of one's self or others and of the group [5]. Goleman advanced ten fundamentals of E.I and they are self awareness, empathy, self-motivation, emotional-stability, maintain -relationship, truthfulness, self- development, value orientation, commitment, altruistic - behaviour.

2. METHODOLOGY

For this study a normative survive method was used to collect data from diabetic physical exercising and diabetic nonphysical exercising people from Chidambaram, Cuddalore district, Tamilnadu, India. This investigate is an attempt to analyse and interpret diabetic physical exercising and diabetic nonphysical exercising people on the psychological aspect of EI. The problem of this study is a comparison of an EI of diabetic physical exercising and diabetic nonphysical exercising peoples. The sample in the present study was limited to 60 nondiabetic and 60 diabetic people.

Anukool [6]. Hyde, Sanjyot Dethe and Upinder Dhar tool are used for measuring EI developed. This scale consists of 34 items with each having 5 substitute choices, this scale has 10. The investigator made home and institutional visits and the data were collected. The instruction is given the manual strictly adhered. Before analysing the data, personal information obtained from the subjects for EI was recorded and presented. The collected data were statistically analysed by t-test was used for the comparison of means of the two sets of scores are significant or insignificant. Additionally, the correlation was implemented to analyse the relationship between the two variables. There were several indications of relationship. In this study Persons rank order correlation was used. The correlation between different variables is found out by the method of product-moment correlation.

3. RESULTS

Table 1 shows the various dimensional scores for emotional dimensional intelligence, such as self-awareness, empathy, self-motivation, emotional stability, managing the relationship, integrity, self-development, value orientation, commitment, and altruistic which collectively accesses the EI. Since the critical value of F is 4.00 for (1 and 118) and the obtained R-ratio value of 14.08, 52.96, 3.30, 5.02, 75.45, 26.57, 7.58, 35.96, 33.75, for Self-awareness, Emotional stability, Managing relationship, integrity, value orientation, commitment, altruistic is larger than critical value so it is decided that there is a substantial change among the diabetic physical exercising and diabetic nonphysical exercising

group in all the dimensions except in the self-development because the obtained value of-1.40 is lesser than the critical value, so there was not at all significant difference found among the diabetic physical exercising and diabetic nonphysical exercising group in the dimension of self-development. And overall, in EI, the obtained r ratio of 3.80 was greater than the critical value so there was a significant difference found between the diabetic physical exercising and diabetic nonphysical exercising group on EI.

Table 1. Presentation the information and the outcome of diabetics physical exercising and diabetic nonphysical exercising for various dimensions of EI

Variables	Diabetic	Number	Mean	Standard deviation	'r' - Value	Significant
Self-awareness	Physical Exercisers	60	17.57	0.06	14.08*	0.00
	Nonphysical Exercising	60	17.22	0.17		
Empathy	Physical Exercisers	60	20.11	0.58	52.96*	0.00
	Diabetic	60	19.33	0.17		
Self-motivation	Physical Exercisers	60	24.87	0.19	3.30*	0.02
	Nonphysical Exercisers	60	24.78	0.02		
Emotional stability	Physical Exercisers	60	16.32	0.01	5.02*	0.00
	Nonphysical Exercisers	60	16.27	0.75		
Managing relationship	Physical Exercisers	60	16.70	0.01	75.45*	0.00
	Nonphysical Exercisers	60	16.41	0.02		
Integrity	Physical Exercisers	60	13.22	0.04	26.57*	0.00
	Nonphysical Exercisers	60	13.18	0.08		
Self-development	Physical Exercisers	60	8.61	0.61	-1.40	0.30
	Nonphysical Exercisers	60	8.62	0.33		
Value orientation	Physical Exercisers	60	7.89	0.10	7.58*	0.00
	Nonphysical Exercisers	60	7.80	0.88		
Commitment	Physical Exercisers	60	8.56	0.01	35.96*	0.00
	Nonphysical Exercisers	60	8.46	0.01		
Altruistic behaviour	Physical Exercisers	60	7.75	0.02	33.75*	0.00
	nonphysical Exercisers	60	7.51	0.04		
EI	Physical Exercisers	60	142.00	0.11	3.80*	0.00
	Nonphysical Exercisers	60	139.42	5.17		

Diabetic physical exercising and diabetic nonphysical exercising group significantly differs in their stress tolerance, because the t- value of 25.86 is significant in 0.05 level. The mean value of 73.81 for diabetic physical exercising people and the mean value of 71.79 for diabetic nonphysical exercising people was less. This indicates that diabetic physical exercising people have more stress tolerance than diabetic nonphysical exercising people. This also indicates that Comparatively diabetic physical exercising people can withstand when deprived and tolerate critics of others because non diabetic physical exercising people are more stress-tolerant compared to diabetic nonphysical exercising people.

Table 2. Showing the result on the basis of diabetic physical exercising and diabetics nonphysical exercising people for the variable stress tolerance

	Diabetics	Mean	S.D	t. Value	LS
Stress Tolerance	Physical Exercisers	73.81,	0.61		
	Non Physical Exercisers	25.86*	0.05	71.79	0.17

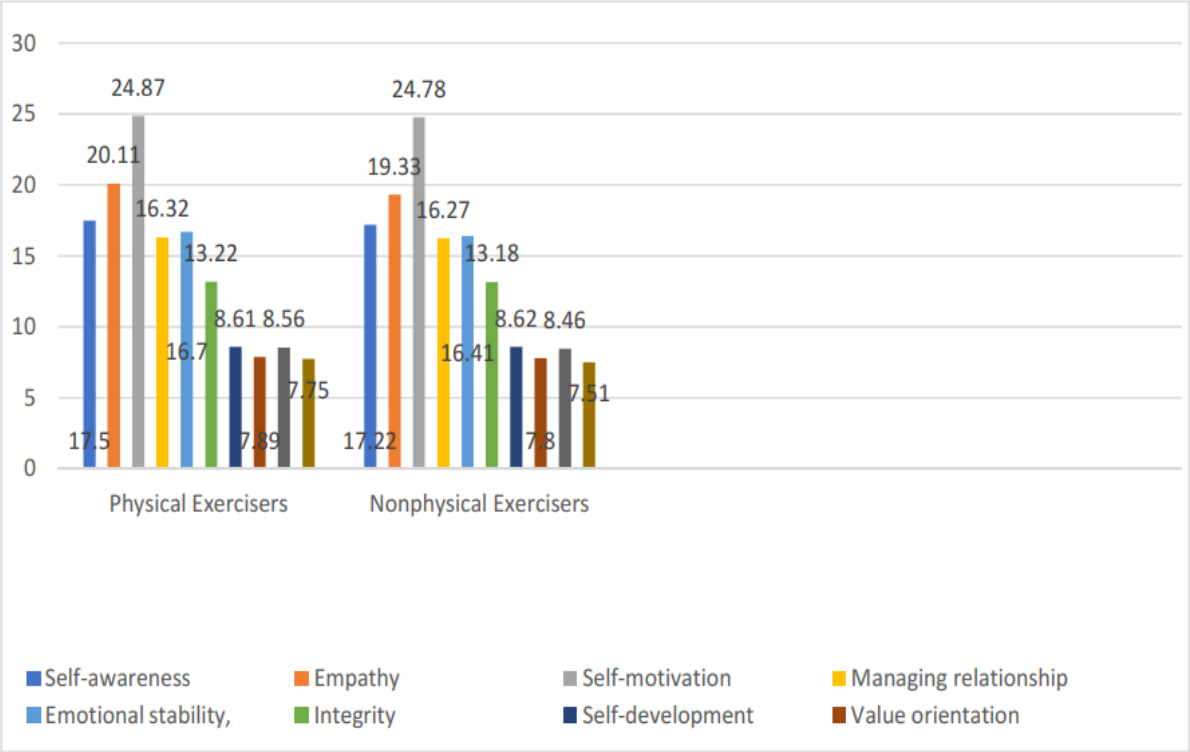

Fig. 1. Presentation the mean value of diabetics physical exercising and diabetic nonphysical exercising peoples

Correlation between various dimensions of EI on stress tolerance of diabetic physical exercising people Correlation under this study was estimated with Pearson's Product moment method Correlation between various dimensions of EI on stress tolerance of diabetic physical exercising people is shown in Table 3.

The result of from (Table 3) shows that the 'r' value obtained from the variable emotional stability and self-development on the sample of 60 on stress tolerance was identified as 0.34 and 0.35 which was significant at 0.01 this shows that there was a positive relationship between stress tolerance and

emotional stability with Stress tolerance and self- development. This show that when emotional stability increases stress tolerance increases, Self-development occurred stress tolerance increases.

The 'r' values of 0.04 self-awareness, 0.56 empathy, 0.10 self-motivation, 0.40 managing relationship, 0.01, integrity, 0.009 value orientation,0 .20 commitment,0.20altruistism, and 0.007 EI are found to be statistically insignificant so this reveals that above mentioned dimensions do not have any relationship with stress tolerance in diabetic nonphysical exercising people.

Table 4 showing the Correlation results of non- diabetic physical exercising people for various dimensions of EI and Stress tolerance.

The result of from (Table 4) shows that the 'r' value obtained from the variable EI on the sample of 60 on stress tolerance was identified as 0.25 which was significant at 0.05 this shows that there was a positive relationship between stress tolerance and EI This show that when EI increases stress tolerance increases for diabetic physical exercising people vice versa.

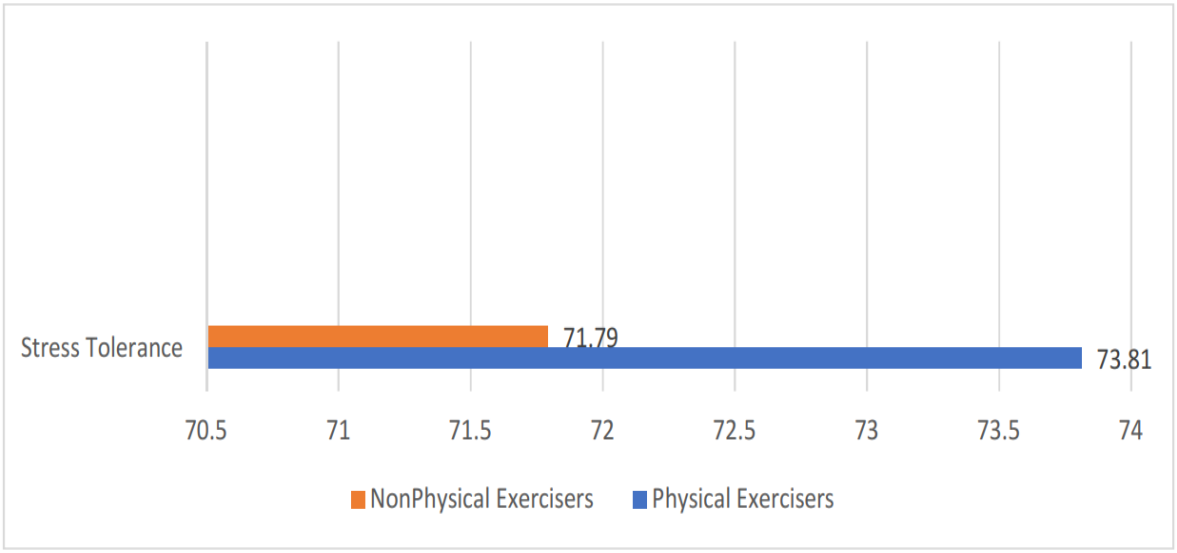

Fig. 2. Showing the mean value of diabetic physical exercising and diabetics nonphysical exercising people for the variable stress tolerance

Table 3. Showing the correlation results of diabetic nonphysical exercising people for various dimensions of EI and Stress tolerance

		Stress Tolerance
1	Self-awareness	0.04
2	Empathy	0.56
3	Self-motivation	0.10
4	Emotional stability	0.34**
5	Managing relationship	0.40
6	Integrity	0.01
7	Self-development	0.35**
8	Value orientation	0.009
9	Commitment	0.20
10	Altruistic behaviour	0.20
11	EI	.007

*** Correlation Coefficient is significant at 0.01 level.*

Table 4. Showing the correlation results of diabetic physical exercising people for various dimensions of EI and Stress tolerance

		Stress Tolerance
1	Self-awareness	0.04
2	Empathy	0.07
3	Self-motivation	0.18
4	Emotional stability	0.10
5	Managing relationship	0.11
6	Integrity	0.07
7	Self-development	0.17
8	Value orientation	0.17
9	Commitment	0.05
10	Altruistic behaviour	0.10
11	EI	0.25*

** Correlation Coefficient is significant at 0.05 level*

4. DISCUSSION

The results of this study showed the score of EI is connected with apparent stress in the experimented subjects that stated that those folks having higher level of EI had higher levels of stress tolerance [7,8]. Findings of current study in line with the findings, with, which confirmed that people who are EI professed had better stress tolerance [9,10,11]. Ample of studies reported that physical Exercise is the best way to be projected on the EI once equated to gender, over-all mood, over-all health and psychological health. Correspondingly, the subscales of adaptable and using emotion were found expressively dissimilar once associated with physical activity group studies supported the relationship between the level of EI and [12]. Exercise training generally causes positive psychological and communal adjustments.

Al Sudani et al. [13] one more study uncovered the detail that has been associated with avoidance, task-oriented coping, social changes with EI is possible through physical exercise [14]. WHO found more vicarious behaviour in persons through higher physical exercise levels. Though, this research is in line with the results concerning the managing relation [15]. Consistent physical activity shown development in psychological wellbeing and self-esteem in diabetic patients [16]. Exercise helps in changing the mood. In line with (NCHPAD) [17]. Humans who participate or practice regular physical activity will not be easily depressed. Physical exercise is the determine way to upsurge stress tolerance and assistances to uphold a sense of emotional wellbeing [9]. In line with diabetes daily.com (2007) overall health that is mental, physical and social wellbeing can be developed through physical exercises.

5. CONCLUSION

There was a positive relationship between stress tolerance with EI which specifies that when EI increases stress tolerance increases for diabetic physical exercising person's vice versa Physical exercises has increased the EI [18]. Stress is one of the primary causes for most of the ailments. So, it is very essential to practice minimum of 45 minutes of exercise which keeps away from stress and ailments.

CONSENT

As per international standard or university standard, respondents' written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Thyroid and Growth Hormones Interdependence and Their Synergistic Effect on Growth and Development at Childhood

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ABSTRACT

Aims: To investigate how variation in the plasma levels of thyroid and growth hormones influences the physical growth indices, and the variation of these hormones with age in children aged 6-10 years.
Study Designed: The study is a prospective study, designed to investigate how variation in growth and thyroid hormones relate to physical growth and development in children.
Methodology: Anthropometric uniqueness were described and Body Mass Index computed for 180 (male 81 and female 99) participants of the study. The children were categorized further based on age into 6, 7, 8, 9, and 10 years. Thyroid and growth hormones were determined by Enzyme-Linked Immunosorbent Assay (ELISA) specific for the various hormones, using STAT FAX 303 microtitre plate reader. Statistical analysis, data management and statistical analyses were performed using SPSS version 23.0 (SPSS, Chicago, IL, USA) statistical package. Difference between groups was determined using Student's t-test, variations among groups by ANOVA and relationship between parameters using Pearson's correlation. Excel was used to chart the relationship between the concentrations of the hormones against the ages of the children. The significance level of the tests was set at α=0.05. Results will be expressed as Mean ± SD
Results: Nineteen percent (n=34) had GH values (≤0.6 ng/ml), lower than the expected normal, 64% (n=115) had values between (0.6-10.0ng/ml) while 17% (n=31) had values ≥10.0 ng/ml. Ninety two percent (n=166) of the children had thyroid stimulating hormone (TSH) values between 0.35-8.44 µIu/ml; 6.1% (n=11) had values >8.44 µIu/ml which are above normal while1.7% (n=3) had values <0.35 µIu/ml lower than normal. Triiodothyronine appears to increase from birth recording a peak within the first year of postnatal life, and then progressively declining to adult values while GH increases from birth attaining a peak about the age of nine then falling progressively to adult values. Children with apparent GH deficiency had significantly higher T3 and T4 levels and lower TSH, those with high GH values had correspondingly low T3 and T4 values and high TSH. The correlation coefficients of TSH and growth hormones in those with high and low GH (r=-0.05 and r=-0.130) respectively were both negative while that of TSH and GH in those with normal GH was positive (r=0.093), however, physical growth indexes are preserved across board.
Conclusion: It was concluded from the study that growth failure may be due to failure of both hormones and may likely not occur when an unbroken synergy exist between thyroid and growth hormones during childhood.

Keywords: Growth; thyroid hormones; interdependence; synergy; growth indices; childhood.

1. BACKGROUND

Atypical growth and development at childhood have been associated with a psycho-social burden and abnormal reproductive adulthood. Growth and development during childhood is regulated by both thyroid and growth hormones.

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1.1 Growth Hormone

Growth hormone is a long single-chain polypeptide hormone made up of 191 amino acids. It is synthesized in somatotropic cells found in the anterior pituitary gland under the tight control of the hypothalamic–pituitary axis and GH secretagogues, the cells also store and release the hormone. Growth hormones are of two types, somatotropin refers to growth hormone formed in animals' growth hormone producing cells while somatropin are growth hormones that are in the synthetic form produced using recombinant DNA technologies [1].

1.2 Growth Hormone Physiologic and Metabolic Effects

The growth hormone is responsible for the regulation of a number of physiological processes such as growth and metabolism.

The physiological effects of growth hormone are of two different types, the direct and indirect effects. The direct effects are observed when growth hormone binds with its receptor on target cells. For instance, growth hormone receptors on fat cells can be stimulated by growth hormones to break down triglycerides, thus regulating the accumulation of circulating fats. On the other hand the indirect effects are mediated by the insulin-like growth factors, also called somatomedins. Several growth-promoting effects of the hormone are due to the insulin-like growth factor acting on its target cells. Its roles include stimulation of development, growth, and regeneration of body tissues [2]. These functions bring about maintenance of normal body structure and metabolism. It also result in building and repair of healthy tissues in the brain and other organs. It contributes to the regulation of body fluids, fat metabolism, sugar and also the functions of the heart. Thus, growth hormone is crucial in the enhancement of growth and development in adolescents and children.

Metabolic effects of growth hormone has critical effects on proteins, carbohydrates and lipid metabolism [3]. The metabolic effect reflects increased protein synthesis and decreased protein catabolism, hence increased muscle mass. Growth hormone stimulates triglyceride breakdown and adipocyte oxidation (lipolysis). Growth hormone preserves blood glucose levels and suppresses the ability of insulin to take up glucose.

1.3 Growth Hormone Secretion and Regulation

Growth hormone (GH) secretion is regulated by a complex neuroendocrine control system that includes the participation of several neurotransmitters and the feedback of hormonal and metabolic substrates.

Growth Hormone is regulated by the releasing hormone called somatocrinin also called growth hormone-releasing hormone along with somatostatin, also called growth hormone-inhibiting hormone (GHIH) or somatotropin release–inhibiting factor (SRIF), which is released by the neurosecretory nuclei of the hypothalamus.

A number of biologic agents including Ghrelin, growth hormone-releasing hormone and sex hormones like estrogen and androgens stimulate the release of growth hormone. Ghrelin acts by binding to Growth Hormone Secretagogue Receptors (GHSR), growth hormone-releasing hormone by linking to GHRHR (Growth Hormone Releasing Hormone Receptor) and estrogen and androgens by stimulation of the secretion of growth hormone at the time of puberty. Growth hormone works on a finely regulated axis, with just the right amount needed to ensure optimal growth of tissues.

Inadequate secretion (hyposecretion) and excess secretion (hypersecretion) of growth hormones has several effects based on the age it occurs in the patient. Hypersecretion of growth hormone causes gigantism in children due to excessive GH and IGF-1 before growth plate fusion and acromegaly in adults, as a result of growth hormone excess after epiphyseal plate closure. Pituitary tumours may also be caused by hypersecretion of growth hormone in adults [4]. Hyposecretion of growth hormone causes dwarfism in children and changes the cholesterol levels (increase fat stores), muscle mass

(reduced muscle mass) and bone strength in adults. Growth hormone is not released continuously by the pituitary gland, but relatively in short pulsatile fashion all through the day. Healthy men produce around 5 ng/mL of growth hormone in the blood. Females produce between 6 to 120 times more so as to preserve an environment suitable for child-bearing, and its relationship to estrogen.

1.4 Thyroid Hormones

Thyroid hormones are synthesized in the thyroid gland by the iodination and coupling of two molecules of tyrosine (an amino acid). The process depends on an adequate supply of iodide. Iodide is rapidly converted to iodine in the thyroid gland, catalyzed by thyroperoxidase. Iodination of tyrosine residues in thyroglobulin results in monoiodotyrosine (MIT) and diiodotyrosie (DIT), mediated by thyroperoxidase. Iodotyrosines are coupled (DIT and DIT) to form thyroxine (T4) and triiodothyronine (T3) (DIT and MIT), which are the product of follicular cells. Before being secreted thyroid hormone still incorporated in thyroglobulin are stored in the colloid of the thyroid follicle where it is taken up by the follicular cells, by a process involving endocytosis and then phagocytosis and T3 and T4 are released by proteolytic enzymes into the blood stream.

The process is inhibited by iodide and stimulated by thyroid stimulating hormone. The enzymatic removal of an atom of iodine from the outer ring of T4 produces T3 the main active form of the hormone because it binds more avidly to thyroid receptor, while removal from the inner ring results in rT3 which is probably inactive

1.5 Thyroid Hormone Actions

Thyroid hormone affects practically each organ system in the body, comprising the heart, CNS, autonomic nervous system, bone, gastrointestinal, and metabolism [5].

Thyroid hormone has general effects via activation of nuclear transcription of several genes, consequently, in nearly all cells of the body abundance of protein enzymes, structural proteins, transport proteins, and other biomolecules are synthesized. The outcome is a widespread improvement in functional activity all through the body. Thyroid hormone controls metabolism, growth, and has many other specific effects in the bodily functions. It activates the genes involved in increasing metabolic rate (increased oxygen and energy consumption) and thermogenesis. One of the primary functions of thyroxine may be purely to increase the number and activity of mitochondria, which consequently increases the rate of formation of adenosine triphosphate (ATP) to energize cellular function. The organ systems affected by thyroid hormone are many, in the heart it has a permissive effect on catecholamines. It increases heart rate, stroke volume, cardiac output, and contractility via increasing the expression of beta-receptors (5). In the lungs, thyroid hormones stimulate the respiratory centers and bring about increased oxygenation as a result of increased perfusion. In skeletal muscles, thyroid hormones cause increased development of type II muscle fibers. Type II muscle fibers are fast-twitch muscle fibers capable of fast and powerful contractions, but when the hormone becomes excessive, the muscles become weakened because of excess protein catabolism [6,7]. On the other hand, lack of thyroid hormone causes the muscles to become sluggish, and they relax gradually after contraction. On metabolism, thyroid hormones increase the basal metabolic rate. It increases the gene expression of Na+/K+ ATPase in diverse tissues leading to improved oxygen consumption, body temperature and respiration rate [8,9]. Thyroid hormones can induce lipolysis or lipid synthesis based on the metabolic status. It stimulates anabolism of proteins but can also induce catabolism of proteins when present in high doses and metabolism of carbohydrates. Thyroid hormones do not modify the blood glucose level, but they can stimulate increased gluconeogenesis, glucose reabsorption, glycogen synthesis, and glucose oxidation. Thyroid hormone has both general and specific effects on growth. During childhood, thyroid hormones act synergistically with growth hormone to stimulate bone growth. It induces synthesis of chondrocytes, osteoblasts, and osteoclasts [10,11,12]. The rate of growth is greatly retarded in those who are hypothyroid, while in those who are hyperthyroid, excessive skeletal growth often occurs, causing the child to become significantly taller at an earlier age. Nevertheless, the bones also mature more quickly and the epiphyses close at an earlier age, and so the duration of growth and the ultimate height of the

adult may actually be shortened. Thyroid hormone promotes growth and development of the brain during fetal life and for the first few years of postnatal life [13].

Without adequate quantities of thyroid hormone, growth and maturation of the brain both before birth and afterward are significantly retarded, and the brain remains smaller than normal [14]. Deprived of specific thyroid therapy within days or weeks after birth, the child without a thyroid gland will remain mentally deficient throughout life. It also improves brain maturation by axonal growth and the formation of the myelin sheath [15].

1.6 Effect of Thyroid and Growth Hormones on Growth

Stunted growth due to growth hormone deficiency had been treated over the past two decades with recombinant human growth hormones. The biochemical changes and clinical symptoms of the effect that this practice has on other hormonal axes had led to the realization of how the GH-insulin-like growth factor-I axis and the adrenal, gonadal and thyroid axes are connected and affect each other. A complex liaison exists between the GH-IGF-I and the Hypothalamic-Pituitary-Thyroid (HPT) axes. The interdependent rapport between growth and thyroid hormones seems to guarantee normal process of growth and development, since abundance of one hormone compensates for the deficiency of the other hormone. The synergy allows preservation of critical developmental processes such as the development of the brain and physical growth indexes even at reciprocal concentrations of these hormones especially in children. GH replacement unmasks central hypothyroidism in 36-47 per cent of subjects with normal thyroid, biochemically, with consequent reduction in the benefit of the replacement plan [16].

Thyroid and growth hormones, apart from their conventional pathways equally have the PI3K (phosphatidylinositol 3-kinase) and MAPK (MAPK: Mitogen-activated protein kinase) non-canonical pathways common to both. The relationship may be initiated from developments in the PI3K and MAPK, common downstream signal transduction pathways for these hormones. Since the pathway uses the same effectors, alteration in gene expression profile is the ultimate outcome; the abundant hormone compensate for the insufficient hormone by engaging the pathway segment common to both in implementing alteration in gene expression. Also, thyroid and Growth hormones demonstrate a range of physiologic functions in metabolism, growth and development common to both [17]. They influence metabolism of proteins, lipids and carbohydrates similarly depending on the metabolic status. The anabolic effect of growth hormones on tissues are exemplified by their influence on increased protein synthesis and decreased protein breakdown; the proteins may serve as enzymes or structural proteins. Growth hormone stimulates IGF-1 mRNA synthesis [18] and therefore, has both IGF-1- independent and IGF-1 mediated actions through which it stimulates growth of skeletal system, and influences the availability of metabolic fuels. The growth promoting actions of GH are principally mediated through production of the insulin-like growth factor (IGF- I), which exert both an endocrine and paracrine effect [19]. Insulin-like growth factor (IGF-1) stimulates the proliferation of chondrocytes in vitro and remarkable decline in IGF-1 levels in young animals corresponds with decreased bone growth [20-22]. The most demonstrated anabolic action of GH growth in children is the stimulation of longitudinal bone, preceding closure of epiphyses. Bone growth is analogous to growth of other tissues and accrues from increased cell number, size, and extracellular ground substance. Longitudinal bone growth is mediated by a complex series of events that occur within the epiphyseal growth plate As long as the epiphyseal growth plates are able to produce chondrocytes, the bones continue to grow and linear growth continues [23,24]. Growth hormone and IGF-1 have corresponding roles in stimulating bone growth, GH stimulates cartilage precursor cells to differentiate and become receptive to autocrine, paracrine and mitogenic effects of IGF-1 [25-27]. In this way GH and IGF-1 promote skeletal growth, maintenance, repair and regeneration. Deficiency of GH in adult results in decreased lean body mass and considerable improvement in muscle mass (weight) and BMI; have been observed upon GH replacement [28-30]. This may be due to the increased protein synthetic and protein conserving ability of GH. The physiological effects of GH on growth and metabolism are achieved through alterations of gene expression profile and synthesis of IGF-1 mRNA [31]. Disruption of GH-IGF-1 axis in mouse models reliably yielded degeneration in parameters of bone health, with a striking decrease in cancellous bone volume, connectivity, trabecular number and spacing [32,33]. Induction of supraphysiological level of IGF-1 in osteoblast causes an increase in bone mineral

density and trabecular bone volume [34]. Excess glucocorticoids affect growth by the lowering of local IGF-I production through IGF-I transcription inhibition, increased rate of apoptosis in growth plate chondrocytes and osteoblast cell lines [35]. As well as increased bone resorption, inhibition of osteoblast activity, and reduced bone matrix production. This leads to growth retardation in children and osteoporosis in adults. The effect can be offset by GH in vitro and in vivo.

Thyroid hormones mediate cell proliferation and together with insulin-like growth factor-1 (IGF-1), insulin and glucocorticoids [17]; they regulate body protein metabolism, thereby, intimately associated with the processes involved in growth and development. Growth occurs through the synthesis of fresh protoplasm from nutrient materials. Caloric restriction and systemic illness obviously restrict growth, as can be deduced from malabsorption syndromes, metabolic acidosis, inflammatory diseases, renal failure and prolonged serious illness [36, 37].

Thyroid hormones have general and specific effects on growth and development. Thyroid hormones affect growth by activation of nuclear transcription of large numbers of genes in practically all cells of the body consequently large number of protein enzymes, structural proteins, transport proteins and other substances are synthesized and available for the body use [38]. The thyroid hormones control early growth and development of most organs especially the brain, and directly manipulate linear growth through stimulation of DNA synthesis in osteoblasts [14].

Growth and development during childhood result from increased size of cell population; determined by the rates of cell proliferation, differentiation and cell death by apoptosis, this is put to check by a system of hormonal mechanism, the key hormone being triiodothyronine (T3) [39,40]. Thyroid hormone has the ability to stimulate the proliferation of IGF-1 mRNA in many tissues and has synergistic effect on GH, specifically in regulation of the growth hormone transcription gene.

GH and thyroid hormones are the major regulators of developmental and childhood growth. Regulations are achieved through their interactions at hypothalamus, pituitary and peripheral tissues such as the liver and most importantly at the epiphyseal growth plate the target organ for convergent hormone action [41, 42]. The effects of hypothyroidism such as reduced growth plate width, articular cartilage, and trabecular bone volume were reversed by combination of GH and T4 in animal models [43].

2. MATERIALS AND METHODS

This study was conducted in Calabar, Cross River State, Nigeria. Ethical approval was obtained from the Research Ethical Committee of the Cross River State Ministry of Health. The co-operations of the heads of the various Schools were obtained and informed consent sought after from parents of the children before being enlisted in the study, an assent was equally given by each child whose consent had been given by the parents. The study recruited 180 (The sample size of 180 was determined using using the formula-

$$\frac{n= Z^2 P (1-P),}{D^2}$$

Where Z=degree of reliability (level of confidence) 95% standard at 1.96, P= estimated prevalence (13%= 0.13), D= margin of error 0.05%, n= required sample size) children in the ages between 6-10 years, attending both private and public primary schools in Calabar. A standard questionnaire was designed and administered to each of the participants. Anthropometric characteristics were described for all the participants and BMI calculated.

A standard venepuncture method was used to obtain three milliliters (3 ml) of venous blood from all the children under aseptic condition; dispensed into plain containers. The samples were allowed to clot and retract then spun at 3000 rpm for 5 minutes. The supernatants obtained were stored frozen till analysis. GH and thyroxin were analyzed by Enzyme Linked Immunosorbent Assay (ELISA) while TSH and Triiodothyronine T3 were determined by Enzyme immunoassay (EIA) and the analyses read using STAT FAX 303 microtitre plate reader; all reagents were obtained from DRG international Inc.

USA. Statistical analysis: data generated in this study were analyzed for level of significance (P = .05) using SPSS (version 20) statistical software. Variations among groups were determined via ANOVA, student's t-test and Pearson's correlation.

3. RESULTS

This research determined growth hormone, thyroxine (T4), triiodothyronine (T3), and thyroid stimulating hormones (TSH) in 180 participants of the study. Table 1 shows physical and biochemical parameter of the various age groups in our study population. Table 2 shows physical and biochemical parameters between those with low, normal and high levels of growth hormones, the compensation for low hormone by the high circulating level of the other hormone resulting in the protection of physical growth indexes among the various groups may be seen. Fig. 1 shows a scatter plot of the means of GH against ages in years, the progressive increase in GH before the attainment of the peak may be seen. Fig. 2 shows a scatter plot of the means of T3 against ages in years; the progressive decrease in T3 towards adult values may be seen. Figs. 3, 4 and 5 show correlations of GH and TSH in children with normal, low and high GH correspondingly.

4. DISCUSSION

Growth and thyroid hormones are the principal hormones concerned with the regulation of growth and development during childhood. They are essential for normal development, growth and metabolism through coordinated actions on different tissues, including liver, adipose tissue, skeletal system and bone [44]. The mean values of T3 and T4 in our locality; 225 ng/dL and 11.34 µg/dL were observed to be higher than 2.20 nmol/L (143.22 ng/dL) and 115 nmol/L (8.98 µg/dL) correspondingly, established in the pediatric reference range, for children in this age bracket [45] (Table 1). This may be due to differences in diet affecting the availability of the precursors of these hormones and their binding proteins. Children generally gain height and weight as they grow from one year to the next, but the biochemical changes that accompany the physical growth are variable.

Table 1. Comparison of physical and parameters of the various age groups in our study population

Age (yrs)	Height (m)	Weight (kg)	BMI (kg/m^2)	TSH (µIU/ml)	T3 (ng/dL)	T4 (µg/dL)	GH (ng/ml)	n
6	1.20±0.01	21.24±0.49	14.57±0.29	4.36±0.56	247±16.0	12.30±0.67	3.01±0.58	29
7	1.23±0.01	23.00±1.32	15.08±0.60	3.28±0.61	244±17.0	12.32±0.76	3.34±1.08	13
8	1.30±0.09	26.05±0.55	15.36±0.23	4.18±0.46	226±11.0	10.98±0.65	5.01±0.79	38
9	1.35±0.01	27.52±0.53	15.20±0.27	4.35±0.39	219±11.0	11.41±0.49	6.09±1.04	48
10	1.40±0.01	31.75±0.62	16.08±0.22	3.68±0.34	214±11.0	10.75±0.48	4.52±0.66	52
p-value	0.000	0.000	0.003	0.548	0.321	0.294	0.127	

Table 2. Comparison of physical and biochemical parameters between those with low, normal and high levels of growth hormones

GH level	Height (m)	Weight (kg)	BMI (kg/m^2)	TSH (µIU/ml)	T3 (ng/dL)	T4 (µg/dL)	GH (ng/ml)	n
<0.6	1.29±0.16	25.38±0.75	14.97±0.27	3.62±0.30	227±11.0	11.82±0.59	0.23±0.03	34
0.6-10	1.32±0.01	27.49±0.53	15.46±0.17	3.94±0.24	235±7.0	11.64±0.32	3.52±0.24	115
>10.0	1.33±0.01	27.52±0.72	15.52±0.32	4.88±0.62	188±15.0	9.69±0.67	14.07±0.94	31
p-value	0.242	0.108	0.318	0.139	0.008	0.018	0.000	

Thyroid hormones economy during childhood is quite inconsistent, a few hours after birth perhaps due to temperature changes plasma TSH increases strikingly in normal newborns, with a peak in the first 24 hours of life. Plasma T4 increases shallowly in return, peaking during the second day of life. These result in consequent increase in T3 in the first year of life. This level of T3 during infancy may likely be the highest in normal life, the level keep decreasing progressively tending toward adult values after

the age of 10 years, as growth hormone (GH) assumes a more dominant physiological role. GH on the other hand increases progressively with peaks occurring at about 9 years, and then beginning to decline to adult levels. This developmental age 9 years, corresponds to the middle of the linear growth period, when GH and IGF-1 play key roles in growth and development (Figs. 1 and 2).

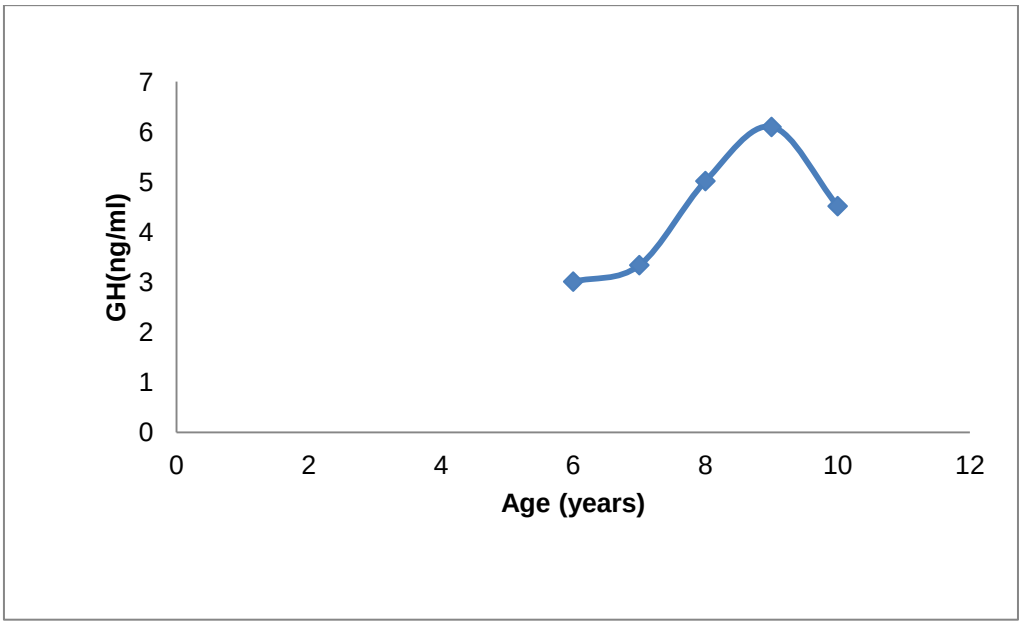

Fig. 1. A scatter plot of the concentration of GH against Ages in years

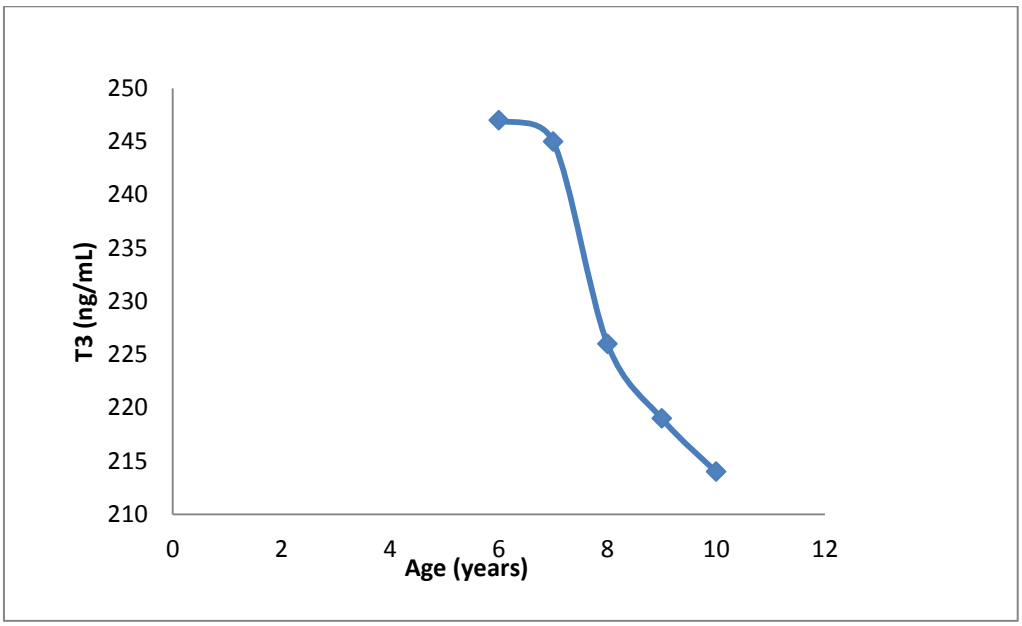

Fig. 2. Scatter plots of the concentration of T3 against ages in years

Thyroid and GH hormones have a converging point in their actions, they stimulate IGF-1 mRNA synthesis in several tissues and this allows them to function in synergy. Their interdependent relationship may be referred to as “reciprocal potentiation” as the physical growth indices among children with low, normal and high GH are not significantly different, the variations in GH being compensated for by corresponding increases or decreases in thyroid hormones (Table 2). Also, the correlation coefficients of TSH and GH in those with low and high GH correspondingly are negative

(Figs. 4 & 5), while that of TSH and GH in those with normal GH, is positive (Fig. 3), however, the physical growth indices are not significantly different across board (Table 2), implying a synergistic effect and a 'reciprocal potentiation' between the hormones. The hormones reciprocally potentiate one another by that interplay between T3, T4, and GH at all concentrations. Children with low T3 and T4 had high GH recompensing for the low T3 and a high TSH working to make more thyroid hormones, while those with low GH had high T3 and T4 and low THS due to the negative feedback inhibition from the high T3 and T4. The interdependency and synergy between growth and thyroid hormones guarantees the preservation of the growth process. These observations are not in line with those of [44], who demonstrated an association of hyperthyroidism with increase means 24-hour serum GH concentration and secretion rates, and [45], who found association between hypothyroidism with an attenuation of GH response to secretagogues and decreased GH pulses. Many potential explanations had been proffered; all in an attempt to elucidate the observed interdependence between the hormones. One mechanism suggested the presence of atypical TRH receptors on somatotropes, perhaps in the pituitaries of acromegalic subjects [46], another proposed an impairment of the inhibitory hypothalamic control of GH secretion [47] or secretion from fetal, neonatal, and prepubertal animals in which the hypothalamic control of GH secretion is presumably functionally immature [48]. Still another suggest changes in the thyroid axis following GH replacement to be associated with complex tissue-specific effects [49]. Downstream signaling pathways for these hormones may be important in the explanation of the synergy between these hormones.

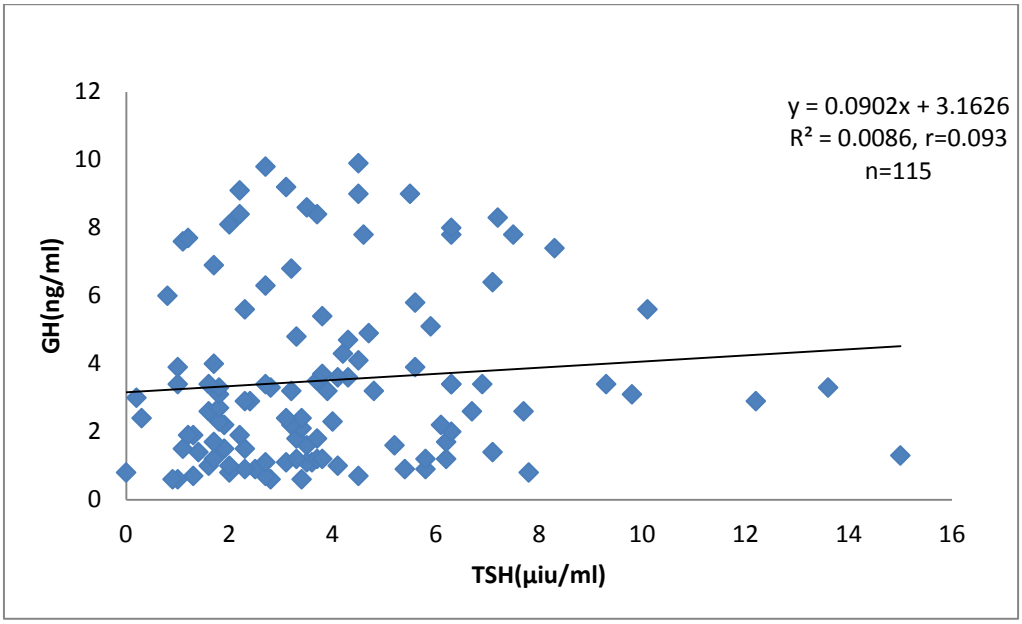

Fig. 3. Correlation of GH and TSH in those with normal GH

Apart from the classical pathways for these hormones they equally have noncanonical signaling pathways that lead to gene transcription. Thyroid hormone action on gene expression now includes nonclassical actions of T3 and T4, T3 has been demonstrated to activate PI3K through the thyroid receptors, eventually increasing transcription of certain genes, also, both T3 and thyroxine (T4) can bind to a membrane integrin, αvβ3, which leads to activation of the PI3K and MAPK signal transduction pathways, finally increasing gene transcription [22]. In spite of the JAK2/STAT conventional pathways for GH action several intracellular signal transduction pathways are activated by GH [50-53]. JAK2 phosphorylation of non-receptor protein tyrosine kinase (src) initiates a cascade of the MAPK pathway with activation of ERK, whereas phosphorylation of insulin receptor substrate-1(IRS-1) triggers the PI3K pathway. Noncanonical signaling independent of JAK2 through src leading to activation of extracellular signal-regulated kinase (ERK) has also been demonstrated. These pathways can then activate signaling effectors such as transcription factors to directly influence gene regulation. The PI3K and MAPK signal transduction pathways are common to both hormones. The "reciprocal potentiation" referred to above may be due to sequential dedication and engagement of

the pathway by the abundant hormone which then yield end products common to the separate actions of the two hormones, thereby reciprocally potentiating one another's effect. Since the pathways lead to alterations of gene expression, the engagement of the pathway by the abundant hormones may have the same net effect of altered gene expression profile that the separate hormones would, when present and acting separately at physiological levels. And by this way the hormones make up for one another's shortfall, the benefit of this rapport is continued normal growth and development in the face of insufficiency of either growth or thyroid hormones.

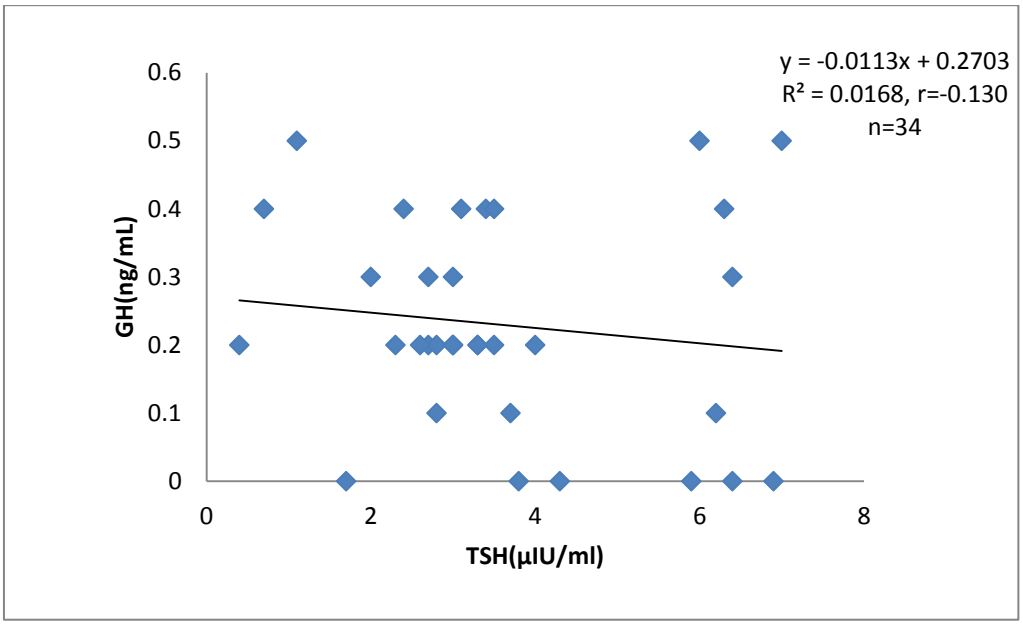

Fig. 4. Correlation of GH and TSH in those with low GH

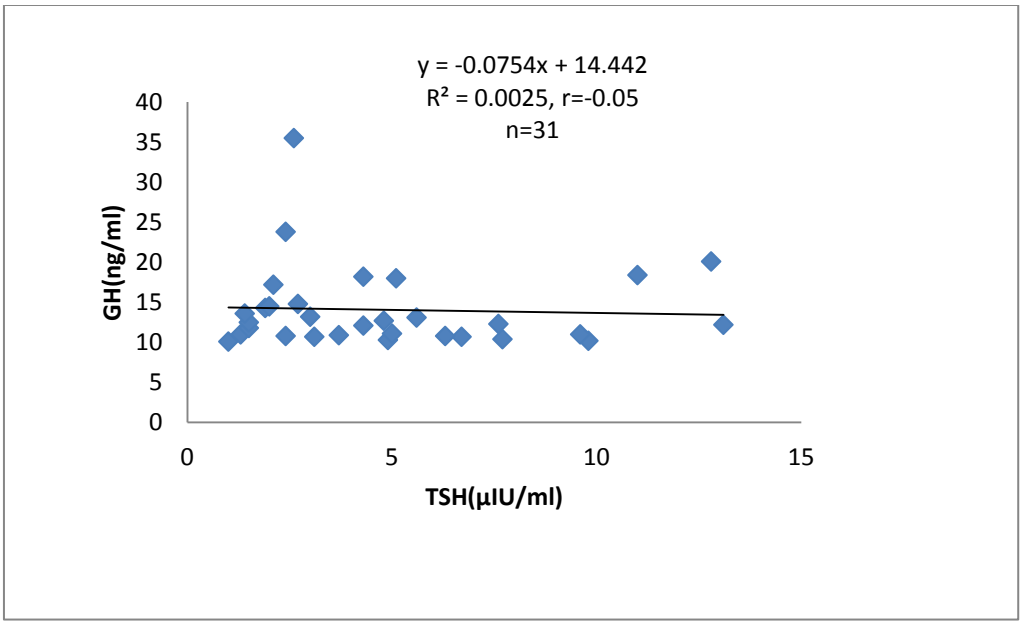

Fig. 5. Correlation of GH and TSH in those with high

5. CONCLUSION

The processes of growth and development in children are salient for normal social and reproductive adulthood, the synergy between growth and thyroid hormones serve as a double mechanism to ensure proper development. Understanding of the molecular mechanisms underlying growth and thyroid hormone actions may have significant implication in human health and diseases. Therefore, in addressing the feasibilities of pituitary hormone replacement; the reciprocal effects that replacement of thyroid and growth hormones may have on the GH-IGF-I and the thyroid axes, respectively should be considered. It was concluded from the study that growth failure due to deficiency of hormones may be due to failure in the secretion of both hormones as may be found in cases of multiple pituitary hormone deficiencies (organic pituitary disease). Growth failure may likely not occur in children with normal pituitary function where an unbroken synergy exist between thyroid and growth hormones during childhood.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Chapter 17
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Analyzing Local Flaps for Coverage of Facial Defects: A Retrospective Study

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DOI: 10.9734/bpi/rdmmr/v10/5005F

ABSTRACT

Background: Face is the center of attention during communication and the expression of emotion. Facial deformities caused by trauma and the excision of skin malignancies are relatively common. The location, size, and underlying cause of the defect, the expected functional morbidity, the patient's medical history, and the practicality of surgery are all factors that influence how this defect is treated. The goal was to investigate various local flaps for defect coverage, outcomes, and complications.
Methods: This was a retrospective cohort study. The results of our study show that 92 patients with facial defects were enrolled in the study from January 2016 to December 2019. Reconstructive options were chosen based on the size and location of the defect. Patients were followed up on for 6 months to a year.
Results: Basal cell carcinoma, squamous cell carcinoma, and melanoma are the most common malignant tumours of the face. Local flaps are always preferred over skin transplants because they produce a better colour and texture match, as well as a vascularized soft tissue cover for the skeleton that is resistant to contractures.
Conclusions: With few post-operative problems, a variety of local flaps were employed to cover the facial abnormalities of the 92 patients in our study.

Keywords: Coverage defects by local flaps; Facial defects; Local flaps of the face; Skin malignancies; Trauma.

1. INTRODUCTION

Surgeons and plastic surgeons commonly use local flaps to fix face defects. Unfortunately, face defects caused by trauma and the excision of skin malignancies are relatively common. Tissue immediately adjacent to or near the primary defect is used to cover the defect in a local flap. Face is the center of attention during communication and the expression of emotion. The location and size of the defect, the expected functional morbidity, the underlying aetiology of the defect, the patient's medical history, and practicality are all factors that influence how this defect is treated. The most common malignant tumors of the face are basal cell carcinoma, squamous cell carcinoma and melanoma [1-3].

While the results of skin graft are less than satisfactory for large areas to cover, distant flaps are bulky with a poor color match. Local flaps provide reasonable option for reconstruction of facial defects with good color and texture match and good success rate. Missing parts should be replaced with similar tissues considering their quality and quantity. For this, the similar flap is always preferable than skin grafts as it produces a superior match in color and texture. It has the additional advantage of producing a vascularized soft tissue cover for skeleton and resistant to contractures. Complications after reconstruction are hematoma, infection, necrosis, deviation site, scar etc. Aim was to study various local flaps using for coverage of defect, outcomes and complications.

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2. METHODS

This was a prospective cohort study done at Department of Surgery, Geetanjali Medical College, Udaipur during January 2016 to December 2019. Our study shows result of 92 patients during January 2016 to December 2019 who had facial defects were taken up for the study. Reconstructive options were selected depending on defect size, location. Follow-up of patients ranged from 6 months to 1 year. All patients admitted in plastic surgery having facial defects in our institute were included in the study. Patients who were operated elsewhere, patients underwent reconstructive surgery other than local flaps, local flaps of various body parts except face.

After taking proper ethical clearance from the ethical committee the current study was conducted.

Sample Size: (n=92) Using Cochrane's formula

to form a rhombus with adjacent angles of 60° and 120°. All sides and the short diagonal of the defect must be equal in length in a 60°-120° rhombus defect and flap. In below image all possible configurations to cover defect are given. Their effective length decreases as they pivot. This reduction in effective length must be considered in designing such flaps [1-6].

$$N \geq \frac{((1-\alpha/2)^2(1-P)}{d2}$$

N= sample size
Z(1-α/2) =Z statistic for 95% confidence interval p= prevalence in view of different literature = 60% d= absolute error=10%

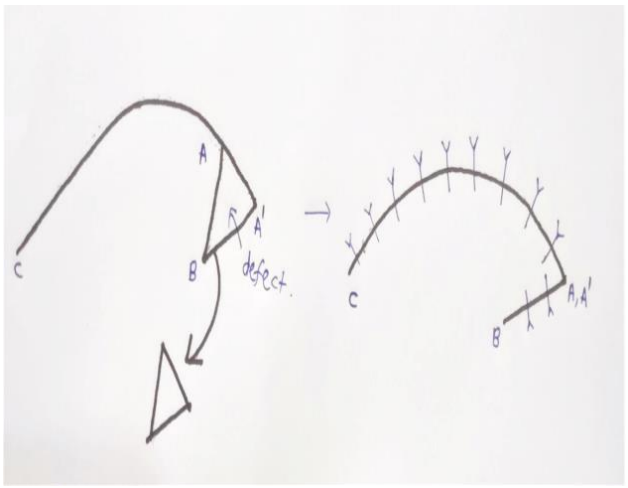

Fig. 1. Rotational flaps

2.1 Statistical Analysis

The data was entered in MS Excel Software version 17 and analyzed using SPSS, IBM Comp, Version 21. The descriptive data was expressed in proportions, mean and frequency tables. The categorical data was analyzed by using Chi-Square test. The quantitative data was analyzed using independent student's T test. P value less than 0.05 was considered statistically significant.

2.2 Surgical Procedure

Rotational flap: They are pivotal flaps with a curvilinear configuration. They are used the most commonly in triangular defects. They are facilitating the pivotal movement of the flap. In below image defect is triangular shape and closed by rotation from surrounding healthy tissue [4,5].

Transposition flaps: They have a linear configuration. They are pivotal flaps moving about a pivotal point. Two types: 1) Rhombic flaps. 2) Bilobe flap.

Rhombic flaps (Limberg flaps): They depend on advancement for part of their tissue pivotal movement. A rhombus is an equilateral parallelogram. 2 equilateral triangles placed base to base

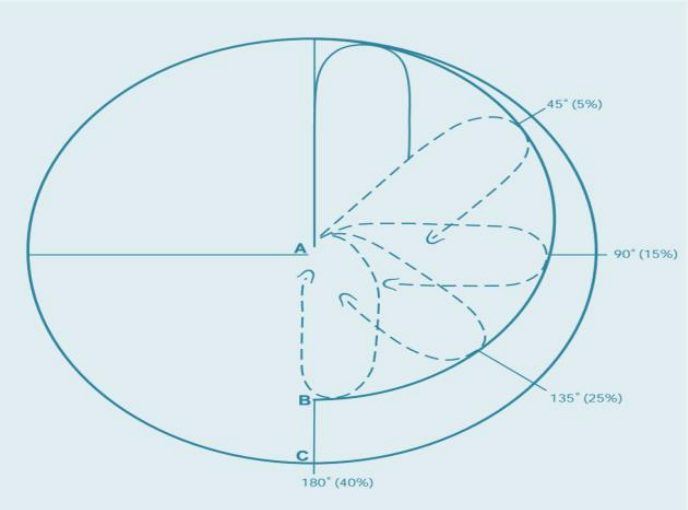

Fig. 2. Transposition flaps

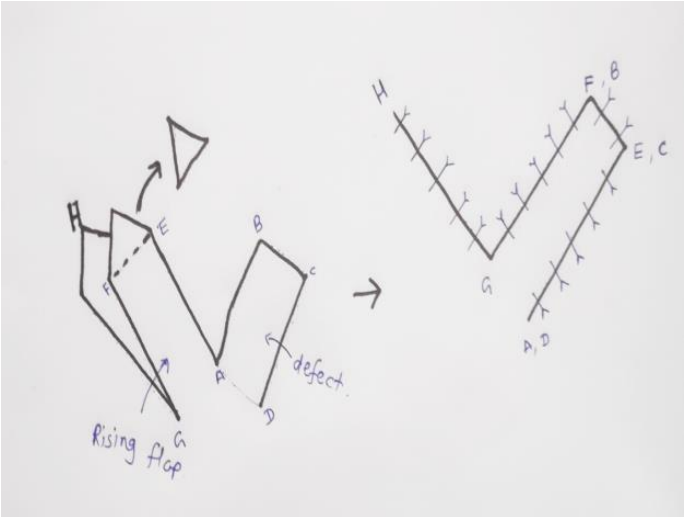

Fig. 3. Rhombic flaps

The bilobe flap: The bilobed flap is a useful and time-honored technique for reconstructing defects of the nose and the eyelid region, especially defects of the lower third of the nose. The bilobed flap is appropriate for partial-thickness losses of less than 1.5 cm of the lateral aspect of the nose, ala, and tip area. This flap is essentially a rotation flap divided into two transposition flaps, with an excellent blood supply from angular and supra-orbital arteries. It recruits skin from the mid dorsum and sidewall. The two flaps have a common base and typically form an arc of no more than 90-110° to avoid tension development on wound closure. The angle between the defect and first lobe is equal to that between the first and second lobe1. The size of the first lobe equals that of the defect, and the second lobe is 2/3 the size of the first lobe. The primary flap closes the defect, and the secondary flap is used to close the donor site. The donor site of the second lobe is primarily closed (Fig. 4).

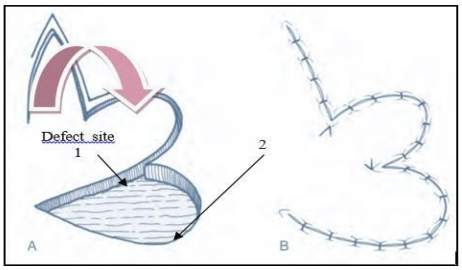

Fig. 4. 1st straight arrow directed movement of flap and 2nd curved arrow directed movement of flap

V-Y and Y-V advancement flaps

Wound closure suture line assumes a Y configuration, with the common limb of the Y representing the suture line. The Y-V advancement flap has a similar principle to the V-Y flap. V-shaped flap is stretched or pulled toward linear incision made at the apex of triangular flap [1,7].

Nasolabial flaps: The superiorly based nasolabial flap is useful for defects of the nasal sidewall, ala, and tip, while the inferiorly based nasolabial flap is useful for defects of the upper and lower lip, nasal floor, and columella. An interpolated design is cosmetically desirable. The blood supply to this flap is excellent due to perforating branches of the facial artery [1]. The color and texture are excellent matches, while the donor site scar is acceptable in the nasolabial sulcus. Using a template defect, a flap is designed on the nasolabial fold. It is best to make the flap exactly match the defect size. The medial incision for the flap follows the nasolabial sulcus, and the lateral incision is placed no higher than the level of the inferior defect margin. The flap is elevated in the subcutaneous plane, and the plane goes deeper as it proceeds superiorly. The flap is rotated counterclockwise on the defect side and transferred to the defect (Fig. 6) [7-11].

V-shaped flap is not stretched or pulled toward the recipient site (defect site) .It achieves its advancement by recoil or by being pushed forward (unique of V-Y). They allow to move into recipient site in nearly tension-free fashion. The defect is repaired with wound closure tension by advancing the two borders of the remaining wound toward each other.

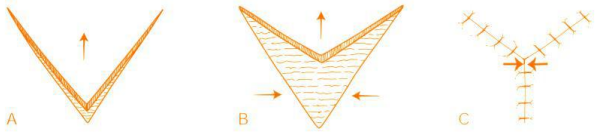

Fig. 5. V Y Plasty

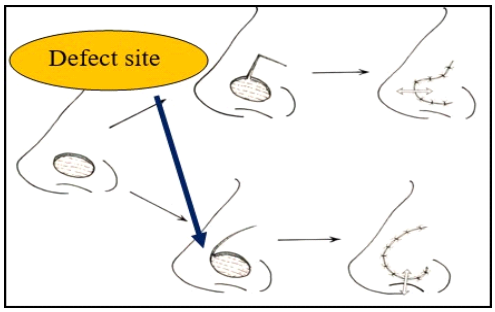

Fig. 6. Nasolabial flaps

3. RESULTS

Our study shows result of 92 patients from January 2016 to December 2019 using local flaps for coverage of facial defects. Out of 92 flaps, 24 flaps were v-y/y-v flaps, 33 were rotation flaps, 12 were transposition flaps used. Two patients developed partial suture dehiscence and two patients developed postoperative hematoma. One patient developed a bad scar over suture line by using bilobed flap and needed revision surgery for that. The W plasty done in that patients and had good outcomes. Postoperative hematoma developed in transposition (Rhombic flap) and rotation flap. But with good antibiotics cover and dressings gave the better outcomes. The facial defects were covered by using limberg flap survived completely without any postoperative complications.

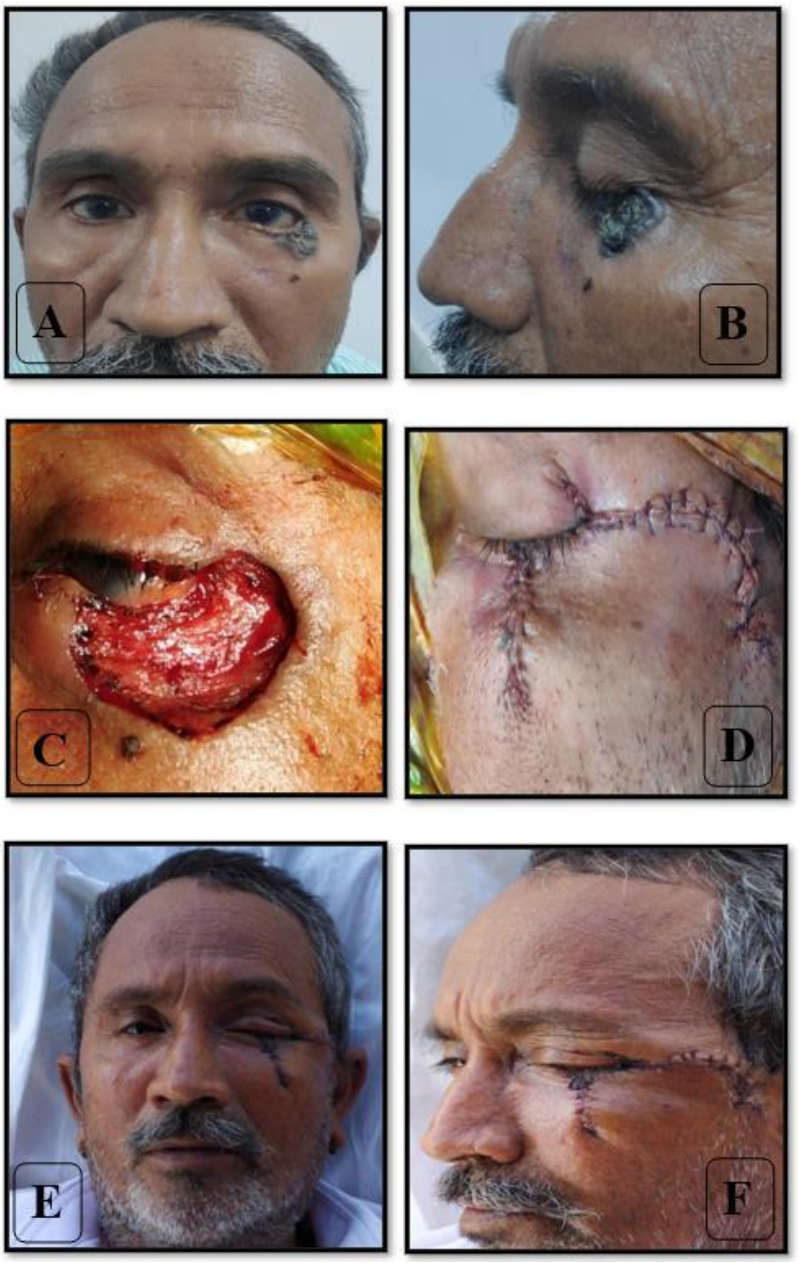

Fig. 7. Post dog bite A) Post dog bite at right labial area and locally arv injected, B) Stay suture of transposition flap, C) Limberg advancement flap covered at defect, D) and E) Front view of face with eye opening and eyes closed

Table 1. Distribution of cases over facial region

Facial area	Male (52)	Female (40)	Total (92)
Forehead	10	13	23
Eyelid and eyebrow	11	7	18
Cheek and nose	23	15	38
Chin	8	5	13
Type of flaps			
v-y/y-v plasty	17	7	24
Transposition (Rhombic flaps)	10	8	18
Rotation	21	12	33
Nasolabial	6	8	14
Bilobe	0	3	3

Any patients had defect near to eyebrow or eyelid by using proper local flaps gave good postoperative outcomes without any angle deviation, eye opening difficulty, epiphora, ectropion, eyelashes, exposure sequel. Eyelid closure was adequate, the margin was well aligned and stable. Cosmetically, no color mismatch was also present (Fig. 7). The one patient had post dog bite facial defect. We gave her ARV with regular doses and ARV at local defect site during operation (Fig. 8).

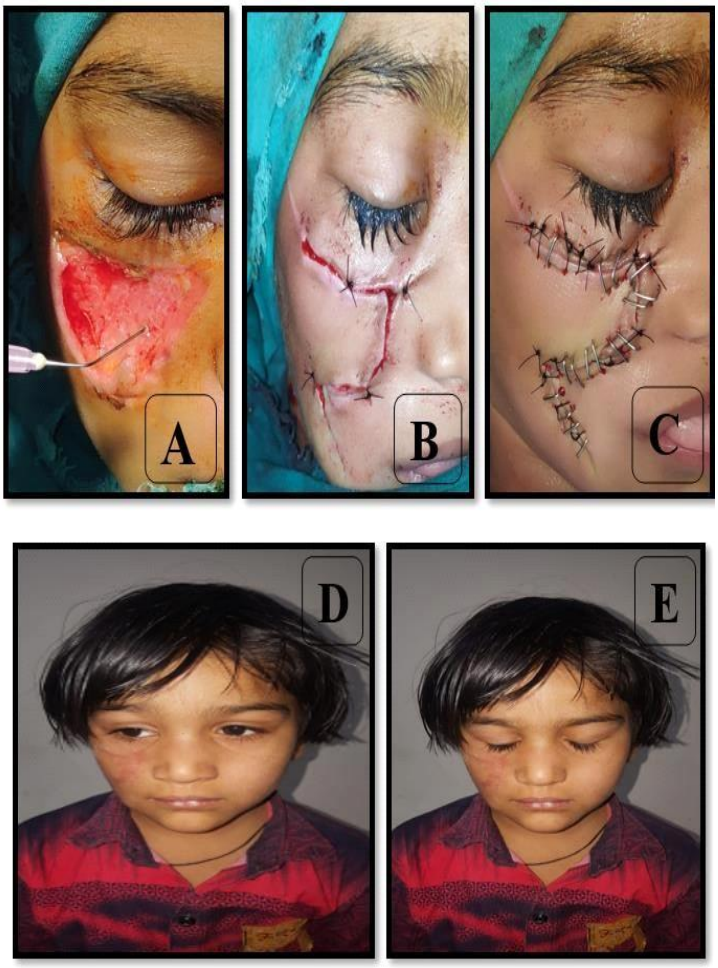

Fig. 8. Post traumatic A) Post traumatic facial defect over right lateral forehead, B) Transposition flap used to cover defect, C) After 1 year at trauma site, cosmetically better results, D) Patient fully satisfied and happy without any complications

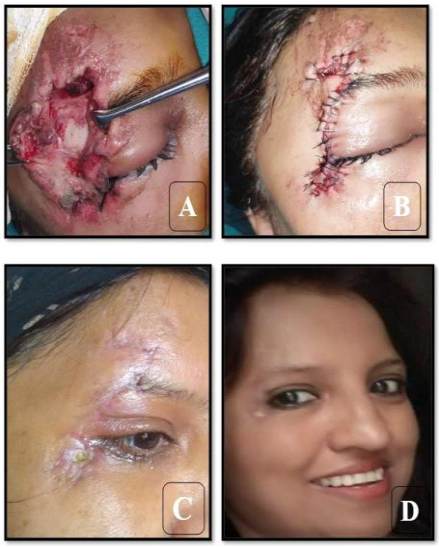

Fig. 9. BCC at nose

Table 2. Postoperative complications of flaps

Type of flaps	Complications	Comments
v-y/y-v plasty	2 Patients developed partial suture dehiscence. 1 patient develop hematoma post operatively.	
Transposition (limberg flaps)	No any obvious complication	
Rotation	Post op hematoma in 1 Patient.	Most common used Minimal post op complication.
Nasolabial	No any obvious complication	
Bilobe	1 patient had post op scar	Need revision surgery W plasty done

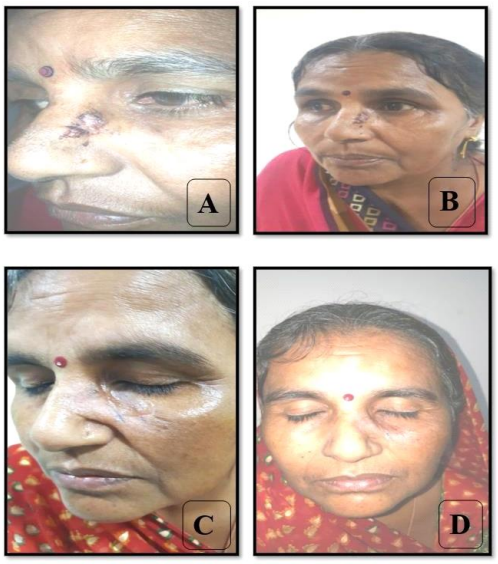

Fig. 9. A) BCC at left nasal wall -lateral view, B) front view, C) Limberg flap used and after 6 months flaps outcomes -lateral view, D) front view

All patients had no anesthesia related complications. We had no post-operative infection or complete necrosis in our patients. All the patients were satisfied after the operation. All flaps survived completely, and there were no any example of flap loss. Follow-up ranged from 6 months to 1year. Tumor recurrence was not seen in any of the patients, during this period. Fig. 6 (BCC at left lower eyelid), A), B) front andlateral view of patient of BCC at left lower eyelid, C) After excision of BCC and made defect, D) rotation flap-suturing after closing defect by rotation advancement flap, E) and F)After 15 days of postoperative no any complication with normal eye opening, no epiphora, ectropion.

Total 92 patients, 71 (77.17%) patients of trauma and 21 (22.82%) patients of skin malignancy. Out of them BCC was 16 patients and 5 patients had SCC.

4. DISCUSSION

The forehead comprises a single facial aesthetic unit but is divided into four reconstructive regions: central, paramedian, temporal, and glabellar. The hairline delineates the superior and lateral borders, while the glabella, eyebrows, and supraorbital rim form the inferior border. The forehead receives a majority of its vascular supply from the internal carotid artery system. Surgeons should be conscious of the course of the facial nerve to preserve the forehead's motor innervation. The nerve travels along Pitanguy's line. The forehead receives sensory innervation from the supratrochlear and supraorbital nerves as well as the zygomaticotemporal and auriculotemporal nerves [7].

Local flaps, especially rotation and advancement flaps, are used in forehead reconstruction. Lateral forehead defects can occasionally be managed with superior advancement of cheek and temple tissue. Rotation flaps are an alternative for moderate-sized defects of the lateral and paramedian forehead regions [12].

From an aesthetic point of view, the cheek may be divided into three overlapping units: 1) suborbital, 2) preauricular and 3) buccomandibular. Zone 1, the suborbital zone, extends along the lateral border of the nose to the nasolabial fold, across the cheek below the gingival sulcus towards the sideburn, up the anterior sideburn to the lateral crow's-foot line and then along the lower eyelid-cheek junction. In this location, wounds not amenable to primary or skin graft closure may respond well to rhomboid, circular or bilobed flaps. In addition, cervicofacial flap design or tissue expansion with rotation from a more lateral site is helpful for larger defects. Zone 2, or the preauricular area, extends from the helical junction with the cheek across to the sideburn to overlap with Zone 1 at the malar prominence. This area includes the tissues over the parotid-masseteric fascia and extends inferiorly to the mandibular angle and lower mandibular border. The local flaps (rotation and transposition flaps) may be used for reconstruction at this location. The Zone 3 is buccomandibular area extending from a vertical division at the middle cheek down to the mandibular margin and from the oral commissure back up to a horizontal division line halfway up the cheek. The transposition flaps may be most useful here [6,10,13,14].

Advancement flap design (based on an incision that allows “sliding” movement of the tissue) is relatively simple and can be successfully applied to repair a wide variety of small or moderate-sized cheek defects. Because the distal end of the V-Y flap is surgically isolated from the donor site, soft-tissue distortion associated with alternative advancement flaps is minimized. The advancing tissue can also be based on a neurovascular bundle. The V-Y advancement flap is equally effective for coverage of large cheek wounds and small defects of those approximating the lid or lateral cheek [6,10].

Reconstruction of the nose is extremely complex. Whenever possible, scar lines should be placed along relaxed skin tension lines. Aesthetic units of the nose need consideration. The nose is divided into nine subunits, which include the dorsum, the tip, the columella and the paired lateral sidewalls, the alar lobules, and the soft triangles. The subunit principle of nasal reconstruction, described by Burget and Menick, advocates the replacement of the entire subunit if the defect involves greater than half of the subunit. This technique disguises scars by placing them within the border of a subunit [15].

The skin covering the bony parts is highly movable while the skin over cartilage parts is thicker, tighter and bound to the cartilage. Healing by secondary intention of convex surfaces like the nose tip should be avoided since healing often is delayed and may lead to uneven scars [7].

The superiorly based nasolabial flap is useful for defects of the nasal sidewall, ala and tip while the inferiorly based nasolabial flap is useful for defects of the upper and lower lip, nasal floor and columella. In the case of defects with diameters between 1.5 cm and 2.0 cm and involving the alar lobules, a nasolabial transposition flap is useful for reconstruction. The nasolabial fold can supply enough skin to resurface the ala, and the contractility of the nasolabial flap can be used to simulate the round, expected bulge of the normal ala [8-11].

The chin presents a reconstructive challenge due to its limited laxity and general intolerance of skin grafts. For large defects, V-Y advancement flap may be used [7].

The most common malignant tumors of the face are basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and melanoma.14 BCC is defined by the World Health Organization Committee on the histological typing of skin tumors as “a locally invasive, slowly spreading tumor which rarely metastasize, arising in the epidermis or hair follicles and in which, in particular, the peripheral cells usually simulate the basal cells of the epidermis” [2].

BCC constitutes approximately 75% of non-melanoma skin cancers. It is usually observed in older patients, especially in those frequently and intensively exposed to ultraviolet radiation during their lives. BCC is often observed in the head and neck areas, especially the eyelid and nose. It is more common in males. The tumor grows slowly. BCC may be treated with surgery, cryotherapy, radiotherapy and curettage and electrodessication [1-2].

Appropriate follow-up after complete BCC excision has been discussed by several previous studies. Park *et al*. report only a 1% recurrence rate after complete excision of BCC and suggest no follow-up of these patients is required [3].

Our study of the ninety patients suggests that local flaps for closure of facial defects due to trauma or skin malignancies give the better outcomes cosmetically than skin grafting and any other reconstructive methods with or without minimal post-operative complications. We most commonly used rotation flaps for facial reconstruction. Our clinical observation suggests various area of face defects covered by specific type of flap which provides good cosmetic and minimal post- operative complications. The details are given below table. We also observed that local flaps participate in the normal facial movements, smiling and speech. Reconstructed structures should simulate the original tissue and the adjacent tissues. Contour, thickness, color, texture, hair bearing and skin elasticity balanced with the adjacent tissues. W plasty and bipedicle local flaps rarely used in face [16,17].

Table 3. Types of flaps

Area	Flaps used	Flaps work well in part of area
Forehead	≥ 1advancement	Mdian and Para-median
	Limberg	Temporal (lateral)
Medial cheek	Limberg	Best used
	V-Y / Y-V plasty	Defects at or below the level of the nasal alae
Lateral cheek	Limberg	Smaller defect
	Rotation advancement	Larger defects
	Nasolabial	Anterior aspect of the alar groove.
Nose	Bilobe	Nasal tip, caudal dorsum, or caudal sidewall.
Eyelid	Rotation advancement	Lateral /upper
	Limberg	Lower eyelid

5. CONCLUSION

In our experience, local flaps give the best results. They are the first choice for reconstruction of the face. They are simple to elevate and less operating time. For facial defects, local flap provides a

versatile and safe alternative. This depends on tissue laxity, vascularity and resulting donor-site distortion. Although many flaps are described, most defects can be best closed by V-Y advancement, transition, rotation flaps. Outstanding functional and cosmetic results can be achieved. Proper execution requires considerable technical skill and experience. Furthermore, a thorough understanding of anatomy and aesthetics is required. With improvements in microsurgical technique and the increased availability of free tissue transfer such as perforator flaps, reconstruction of facial defects has recently undergone rapid evolution. However, the local flaps are still the workhorse for facial reconstruction, placing them at prior step in reconstruction ladder.

6. LIMITATION

For very large fascial defect this flap is not useful. No skin grafting or free flap has been used in this study.

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 18
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Uterine Non-Hodgkin Lymphoma: A Rare Gynaecological Presentation

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ABSTRACT

Primary extranodal non-Hodgkin lymphomas of the genital tract are rare, and engagement of the uterine corpus is not only extremely rare but also lacks pathognomonic signs and symptoms, resulting in delayed diagnosis and treatment. This prompted the publication of this case report of a multiparous woman who presented with recurrent vaginal bleeding and progressive abdominal swelling that was initially suspected to be uterine sarcoma. She had a surgical operation twice, which was initially inoperable in a secondary facility, and subsequently had a total abdominal hysterectomy and bilateral salpingo-oophorectomy and omentectomy in a private facility by a gynaecologist and general surgeon. Specimens removed were sent for histopathological analysis and the diagnosis of high-grade large B-cell type non-Hodgkin lymphoma of the uterus was made. However, her condition deteriorated further, culminating in her demise on account of multiple organ failures before the commencement of chemotherapy. This report also emphasized the difficulties in diagnosis and management in a resource-limited setting where advanced diagnostic aids are not readily available. Although uterine non-Hodgkin lymphoma is uncommon, it should be considered in any patient who has an abdominal/pelvic mass and/or abnormal vaginal bleeding.

Keywords: Non-Hodgkin lymphoma; uterine; abnormal vaginal bleeding; extra-nodal lymphoma; case report.

1. INTRODUCTION

Non-Hodgkin lymphomas (NHL) are haematological malignancies of lymphatic cells. Primary nodal non-Hodgkin lymphoma develops from lymphatic organs such as the lymph nodes, spleen, or Waldeyer's ring. Non-lymphatic tissues/organs, such as the liver, kidney, thyroid, skin, and gastrointestinal system, may potentially be the source. They're known as extranodal in this scenario [1]. Though there are controversies surrounding the classification of NHLs with extra lymphatic involvement, resulting in variability in reported incidence from various studies, extranodal NHLs are not as common as primary nodal NHL and commonly involved sites include the gastrointestinal tract, bone, testis, salivary gland, thyroid, liver, kidney, and adrenal glands [2-3]. Available pieces of literature indicate that primary extranodal NHL of the female genital tract is very uncommon and the majority do not involve the uterine corpus, the ovary, and uterine cervix being the more commonly affected sites [2-4] Also, the most common histologic subtype in developing countries is the diffuse large B-cell lymphoma (DLBCL) as opposed to follicular lymphoma in developed countries [2,3,5]. We present the report of a 40-year-old P2+4 lady who presented at our facility with 8 months history of recurrent bleeding per vagina and 4 months history of progressive abdominal swelling. She was

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initially suspected to be a case of uterine sarcoma, but histology findings of samples from the tumour were in keeping with uterine lymphoma.

2. CASE REPORT

A 40 years old P2+4 Nigerian lady initially presented on referral to our facility in June 2020 following 8 months history of recurrent bleeding per vagina and 4 months history of progressive abdominal swelling and weight loss. At the onset, bleeding was spontaneous and copious with the passage of clots and associated dizziness but no loss of consciousness. Abdominal swelling started as small suprapubic growth which subsequently progressed rapidly to involve the entire abdomen with associated obstructive symptoms like constipation, oliguria, bilateral hydronephrosis on ultrasound and CT scan, and significant weight loss but no fever or night sweats. Her last confinement was about 9 years before the presentation. There were no untoward findings in her gynaecological as well as her past medical, social, and family history. She had initially presented to the referring hospital on account of her symptoms and she had multiple blood transfusions on account of recurrent anaemia during her evaluation. Abdominopelvic CT scan done revealed multiple uterine masses with ascites and suspicion of malignancy while chest CT scan was normal. She was scheduled for exploratory laparotomy and a sample from the uterine mass was obtained for histology as it was adjudged unresectable. Histology report revealed sheets of neoplastic lymphocytes with peripheral chromatin distribution having irregular notched nuclear membranes and several nucleoli. Findings were in keeping with non-Hodgkin lymphoma of the high-grade large B-cell type. Findings on examination at her initial presentation to our facility were of a chronically ill middle-aged woman with notable pallor and about 32 weeks size abdominopelvic mass which was firm in consistency, irregular, non-tender, not attached to the overlying skin, can get above but not below it. Speculum examination revealed a foul-smelling exophytic mass projecting from the cervix with egress of blood into the posterior vaginal fornix. Chest X-ray done revealed no significant abnormality, viral screening (HIV, HBsAg) were negative, tumour marker (CA-125) was not remarkable. She was admitted, resuscitated, and received care from a multidisciplinary team of gynaecologists, haematologists, and medical oncologists. She had multiple blood transfusions on account of recurrent anaemia. She was requested to have immunohistochemistry done on the sample from the prior surgery, but this could not be done until an industrial action disrupted clinical activities, resulting in the patient seeking care in another facility where she had a total abdominal hysterectomy and bilateral sapling-oophorectomy and omentectomy done with intraoperative findings of massive ascites and huge mass involving the uterus, ovaries, and fallopian tubes with multiple seedlings on the bowel. Excised tissues were again sent for histology in a second and different laboratory and this was equally histologically diagnosed diffuse large B-cell non-Hodgkin lymphoma. She however presented to our facility again at about 2 weeks post repeat surgery with rapidly progressive abdominal swelling with obstructive symptoms (constipation, oliguria, and ultrasound findings of bilateral hydronephrosis) and multi-organ dysfunction. Multiple firm abdominopelvic masses were palpated on examination. A foul-smelling exophytic mass extending to the lower third of the vagina with contact bleeding was palpated on digital vaginal examination. She was readmitted and had multidisciplinary team management. Serial renal function studies showed uraemia and elevated serum creatinine which was managed by the Nephrologists. A repeat abdominopelvic ultrasound scan showed multiple intra-abdominal masses with ascites (Figs. 1b, 2) and grade 3 bilateral hydronephroses while CT scan revealed large intra-abdominal masses, bilateral hydronephrosis, multiple peritoneal nodules, periumbilical (sister Mary Joseph) nodules, and left pleural effusion with lung collapse seen in (Figs. 1a, 2- 3). She had ultrasound-guided nephrostomy tube insertion by interventional radiologists and had chest tube insertion by the cardiothoracic surgeons. She was considered for ultrasound-guided abdominal paracentesis, but this could not be done as a repeat abdominopelvic ultrasound finding revealed multiple echogenic masses with loculated fluid in different pockets. She was being optimized for chemotherapy and requested to run an immunohistochemical analysis of the excised sample. This was, however, not achieved as her condition deteriorated further, culminating in her demise on account of multiple organ failures.

3. DISCUSSIONS

Non-Hodgkin lymphomas are the most common lymphoproliferative disorders and be a leading cause of cancers in Sub-Saharan Africa [6]. Akinbami et al reported it as the third most common

haematological malignancy accounting for about 14.6% of all haematological malignancies seen in a tertiary hospital in Lagos, Nigeria [6]. It is also 2 to 3 times as common as Hodgkin's lymphoma [6-7]. They may be classified based on the clinical-grade or histologic subtype [1,5,8]. Though there are many classification systems, the generally popular ones include the Ann Arbor classification, Harris and Sully modification, and the Lugano classification, the latter two classification systems having emanated from the former [1,2,8,9]. They are classified as "primary nodal NHL" when they arise from lymphatic tissues like the lymph nodes, spleen, and thymus and classified "primary extranodal NHL" when they arise outside the lymphatics, like the gastrointestinal tract, salivary gland, bones, thyroid, skin, liver, and the genital tract [2-5]. However, there are controversies in the nomenclature and, thus, the classification when there is the involvement of lymphatic sites with significant extra lymphatic tumour deposits, depending on whether strict criteria or liberal criteria are used for the classification [10]. Although this has a significant influence on the reported incidence of the different types of NHL, primary extranodal NHL is not as common as primary nodal lymphoma [3,4]. In a study of 100 cases of NHL by Oluboyede *et al* in Ibadan, Nigeria only 3 were primary extranodal NHL [7]. Histologically, diffuse large B-cell non-Hodgkin lymphoma is the commonest subtype accounting for up to 42.5% of cases in developing countries as opposed to the developed countries where follicular lymphoma is the commonest findings with an incidence of about 25.5% [2,5,6]. Mean age at diagnosis of NHL in Nigeria is 32.3±16.3 years while the median age was 29±16.3 years [11]. In the Harris and Scully series of 25 cases of non-Hodgkin lymphoma of the cervix, body, and vagina, the disease was noted to affect all sections of genitalia indiscriminately and the age of the patient ranged from 20 to 80years, however, had a predilection for the forties [12-15]. In another study of 43 cases by Muntz *et al*., 77% had uterine non-Hodgkin Lymphoma before menopause [15]. Studies from our facility, which is the immediate environment of this patient, revealed that the mean age at presentation was 38.67±14.82 years while the median age was 34.50±14.82 years and our patient's age of 40 years is in keeping with these findings [6]. This is also in consonance with other studies that have shown that the median age at diagnosis is lower for females in developing countries (42.9 years) than in developed countries (48.9 years) [16]. There is a male predominance of NHL, especially in developing countries [6,11]. However, while Perry et al reported a 57% male predominance in developing countries (vs. 51.1% in developed countries) [5]. The male predominance in developing countries has, however, been attributed to sex inequality in accessing care which disenfranchises the womenfolk [17]. Primary genital NHL is rare, accounting for about 2% of all extranodal NHL [2]. It commonly involves the ovaries and uterine cervix but involvement of the uterine corpus is very rare, with a reported incidence of about 0.5% of all extranodal NHL [2,18]. Also, the NHL of the uterine corpus may be difficult to differentiate from other uterine neoplasms as they do not present with any pathognomonic symptoms [4]. They may be asymptomatic in the early stages and may subsequently present with an abdominopelvic mass or abnormal uterine bleeding [3]. The "B symptoms of lymphoma, like fever, night-sweat, and unexplained weight loss may be absent, posing a diagnostic challenge and resulting to delay in making the diagnosis [19]. In the current case, the patient had presented with recurrent bleeding of about 8 months duration which she was misconstrued for irregular menses. She was essentially stable at the early phase of her illness, but bleeding became more pronounced, necessitating recurrent transfusions. There was also superimposed abdominal mass initially misdiagnosed as uterine fibroids. Possibility of uterine sarcoma was made on further assessment, considering the rate of tumour progression but uterine lymphoma was not suspected until after tissue diagnosis, partly because this patient had no peripheral lymphadenopathy and the only "B" symptom present was weight loss which can also be seen in uterine sarcoma and other malignancies. Because the single most important prognostic indicator for NHL of the genital tract is the Ann Arbor stage, early diagnosis and management are necessary [20] However, it is important to have accurate evaluation and response assessment before the commencement of definitive treatment [1,20]. Immunohistochemistry (IHC) is important for accurate tumour diagnosis, disease classification, and management. The authors recognized that a low percentage of 27.6% of lymphoma diagnosed by IHC were diagnosed correctly by Hematoxylin & Eosin (H&E) and morphology alone [16,21]. While histology remains the first-line diagnostic approach, the histological complexity of lymphoma is a drawback in making a proper diagnosis. Differentiating reactive lymphoid lesions from malignant lymphomas may be difficult without IHC. This brings to the fore the inadequacy and inefficiency of the use of histology alone to make a diagnosis of NHL. This is an obvious limitation of this case report. However, reliance on two histology reports from different laboratories could confirm the diagnosis of non-Hodgkin's lymphoma. Because of its rarity and lack of specific presentation, primary genital

lymphomas including uterine NHL are not only difficult to diagnose but there is also no consensus on modalities of treatment [4]. While treatment is individualized, chemotherapy and radiotherapy have been the mainstay of management and lymphomas are chemo- and radiosensitive, while recent data favoured radiotherapy only, to achieve excellent cure rate in cases of extranodal, localized, low-grade non- Hodgkin's lymphoma, and chemotherapy only for excellent cure rate in young women with non-Hodgkin's lymphoma to preserve their reproductive function and prevent the micrometastasis [12]. Either of the two treatment options had been combined with surgery. Aggressive management is advocated in cases of locally advanced and/or high-grade lymphoma as seen in the case presented [22]. Limiting the role of surgery especially in advanced disease prevents the surgery from aid tumour dissemination [4]. This patient deteriorated clinically and presented with a bigger abdominopelvic mass and left pleural effusion barely two weeks following extensive abdominopelvic surgery for her malignancy. This might have played a major role in her decline and subsequent demise.

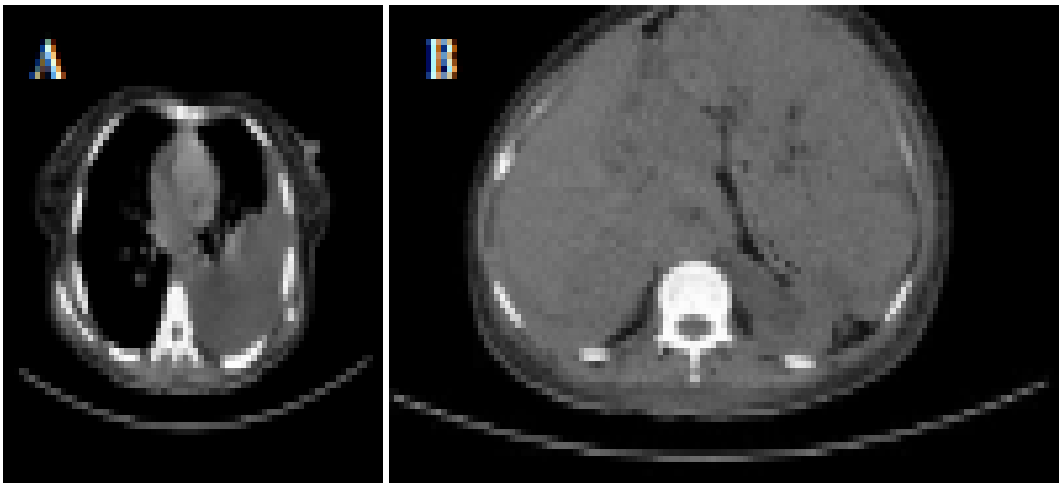

Fig. 1. A) axial thoracic CT, showing the left pleural effusion with left lung collapse, B) axial abdominopelvic CT, showing perihepatic ascites and left hydronephrosis and hypovascular masses

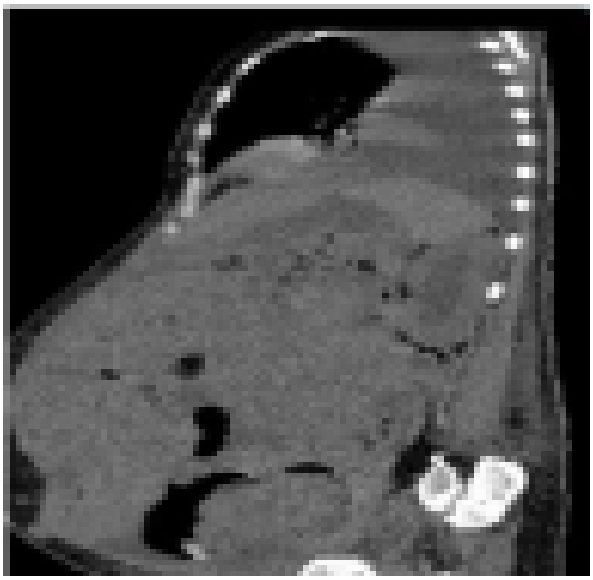

Fig. 2. Sagittal section of the thoracic and abdominopelvic CT, showing multiple intraabdominal masses, ascites, and left pleural effusion

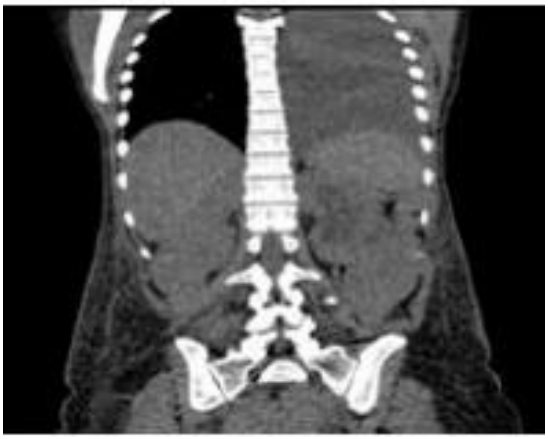

Fig. 3. Corona section of the post-operative thoracic and abdominopelvic CT, showing multiple intraabdominal masses and left pleural effusion

4. CONCLUSION

Uterine NHL is a rare tumour with varying presentations. Fatality is influenced by the tumour stage, histologic type, and poor management. Prompt/accurate diagnosis and appropriate management are pivotal to patients' survival. It should, thus, be a differential in any patient presenting with an abdominal/pelvic mass and/or abnormal bleeding per vagina.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Determining the Factors Associated with Prognosis of Non-Alcoholic Fatty Liver Disease

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ABSTRACT

Background: At the junction between obesity, metabolic syndrome and liver failure, lies Non-alcoholic fatty liver disease. Recent studies elaborated on role of metformin in patients with non-alcoholic fatty liver disease. This observation has not been studied at a global scale, neither it was investigated in different ethnical groups.
Objectives: We aim at determining the risk factors associated with prognosis of non-alcoholic fatty liver disease among a cohort of patients in Southern West Bank, Palestine.
Methods: A retrospective cohort study involving 300 NAFLD patients who visited the internal medicine department at Hebron Governmental Hospital from October 2017 till September 2018. Two hundred and three patients diagnosed with non-alcoholic fatty liver disease, were included in this study. Lab test results within the past 6 months, comorbidity and medication history were collected from patients` profiles. Data was analyzed using SPSS V20. Liver Fibrosis score was determined by using non-alcoholic fatty liver disease fibrosis score calculator.
Results: Two hundred and three non-alcoholic fatty liver disease patients (58.6% females), 54.78 (±12.27) years old were included in the study. Almost 65.5% of these patients have BMI >30 Kg/m^2. It was found that, 62.25% of the 58 diabetic patients in this study had liver fibrosis score > 0.676 comparing to non-alcoholic fatty liver disease patients who are non-diabetic. There was a significant relationship between diabetes and fibrosis score, α=0.000. There was also a significant relationship between hyperlipidemia and fibrosis score of non-alcoholic fatty liver disease patients, α=0.023. We found a significant relationship between fibrosis score and hypertension, α=0.000. In the same context, there was a significant relationship between NAFLD patients who were on statin therapy and those who were not using statin therapy, α= 0.015. Metformin was not associated with significant relationship between users and non-users non-alcoholic fatty liver disease subjects.
Conclusion: Diabetes mellitus, hypertension, hyperlipidemia and statin use were associated with NAFLD prognosis. Tight control of hypertension and dyslipidemia are mandatory among all NAFLD patients.

Keywords: Liver fibrosis score; hypertension; dyslipidemia; diabetes mellitus; metformin; non-alcoholic fatty liver disease.

ABBREVIATIONS

ACEIs : Angiotensin-converting-enzyme inhibitors,
CCBs : Calcium channel blockers, BMI: Body Mass Index.

1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is considered as the first cause of end- stage liver disease in Western countries [1]. It occurs as a consequence of the accumulation of fat in hepatocytes without

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significant alcohol consumption. The American Association for the Study of Liver Diseases in 2018 defined NAFLD as the presence of 5% hepatic steatosis without evidence of hepatocellular injury in the form of hepatocyte ballooning [2].

The prevalence of NAFLD worldwide is thought to be on the rise over the next 20 years [3]. Global prevalence of NAFLD disease varying between 20-50% [4-7]. NAFLD disease was recorded the highest prevalence in the Middle East and South America (31.79% and 30.45%), respectively, while the lowest attribution was reported in Africa (13.48) [8]. Ultrasonography survey in the Mediterranean region indicated that the prevalence of NAFLD was 36.8% in men and 25.7% in women [9]. A study on general population in 2006 indicated that the prevalence of NAFLD in Israel was 30% [10].

The pathogenesis of NAFLD is associated with a variety of complex and multifactorial pathological conditions known as Metabolic Syndrome [11,12]. Insulin resistance plays a central role in the development and progression of NAFLD [13,11]. Insulin resistance cause hyperinsulinemia which results in the development of steatosis, hepatic denovolipogenesis, and increased adipose tissue lipolysis. This leads to rising in the level of free fatty acids and consequently increased fatty acids in the liver [11,13,14]. Once in the liver, free fatty acids causes a chronic low-grade inflammation. This will provoke in turn an inflammatory response mediated by immune cells, chemical mediators and adipocytes leading to disease progression and liver damage [15].

Age, gender, obesity, body mass index (BMI), disease state and other concomitant diseases such as Diabetes Miletus, hypertension, dyslipidemia are risk factors contribute to the development and/ or progression of NAFLD [16-33]. These factors affect to various degrees the prognosis of the disease.

Life style of patients such as smoking or stagnant life style, affect NAFLD prognosis too. Many studies considered moving time versus setting time as risk factors in developing and progression of the disease [34-41].

Controversial reports were found about role of various Anti-hyperglycemic medications (metformin, insulin, sulfonylureas) in NAFLD. In addition to that, antihypertensive and lipid lowering agents (statins) play major role in NAFLD [42-77].

Statins are the most widely used lipid lowering agents in dyslipedimia [74]. They reduce cholesterol levels by inhibiting HMG-CoA reductase enzyme [75]. According to dose and type, they lower LDL cholesterol by 20 to 60%, triglycerides by 10 to 33% and increase HDL cholesterol by 5 to 10% by average [76]. It is approved to decrease liver fibrosis in NAFLD patients where it is effective and safe option [75]. Atorvastatin has been played a positive role in delaying lipid deposition in patients with NAFLD, but the overall effect is limited [78]. Current international guidelines are not recommending statins for the management of NAFLDs patients and suggest that more biopsy-proven benefits are mandatory from large randomized trials [79].

In this study we are going to evaluate for the first time in Palestine the factors associated with NAFLD prognosis and risk factors for developing terminal liver injury while looking for positive factors that might improve it.

2. METHODS

A retrospective cohort study involving all NAFLD patients who visited the internal medicine department at Hebron Governmental Hospital between October 2017 and September 2018 was done. We reviewed 3000 patients` electronic and/or paper-based profiles during that period. A face to face or telephone-based interview with the patient or his/her caregiver was done when necessary in order to get precise information or missing information from profile. Only 203 NAFLD patients were included in the study who have their laboratory test results for ALT, AST, IGF, platelet count, and Albumin done within the past 6 months. SPSS version 20 was used to analyze the data. NAFLD fibrosis score was determined using NAFLD fibrosis score calculator by Angulo P. et. al, available on line.

3. RESULTS

As shown in Table 1, 203 patients were included in the study, (58.6% females), age (54.78 ± 12.27 years old). Most of them, (50.2%), were in the age group of 40-59 years and 76.8% of them were non-smokers. Majority of patients were living in villages, (59.6%)

In fact, 65.5% of subjects were obese, (BMI >30 Kg/m2). For daily activities, 39.9% of patients have a sitting time > 7 hours per day while 37.4% had moving time from 1-3 hours. For meals; 54.7% of them had 2 meals per day while 34.5% had more than 3 meals per day.

In addition to NAFLD, we found that 119 subjects suffered from various comorbidities. Fibrosis score as main outcome of the study was calculated for all patients and they were categorized accordingly as shown in Table 1 below.

As shown in Table 2 below, there was a significant difference in fibrosis score between NAFLD patients who have diabetes and NAFLD patients without diabetic, α =0.000. There was no significant difference between the 2 groups according to years of diabetes, α=0.167.

Dyslipidemia was a major factor in prognosis of NAFLD. We found a significant difference between patients who have hyperlipidemia and who had not, α=0.023.

There was also a significant difference in fibrosis score of different patients` categories and hypertension, α= 0.000

We also studied the effects of medications on NAFLD prognosis and fibrosis score. As shown in Table 3 below, there was no significant difference between patients categories and the following independent factors; metformin, insulin or sulfonylurea use, α values were 0.975, 0.706 and 0.393 respectively.

Regarding anti-hypertensive agents; there was no significant difference between using antihypertensive agents; Beta-blocker, ACEI or CCB and fibrosis score, α values were 0.413, 0.182, and 0.304 respectively.

Concerning use of anti-hyperlipidemic agents, there was a significant difference between patients categories and anti-hyperlipidemic agents (statins), α=0.015

4. DISCUSSION

Insulin resistance and adipose tissue dysfunction which occur as a result of imbalance of adipokines (such as leptin and adiponectin) secretion [27], are the main contributing factors relate obesity to NAFLD rather than fat accumulation [28]. J. M. Clark et.al, (2002) reported that NAFLD occur in 30% of obese men and 40% of obese women [29].

Our results come in agreement with the previous report by J.M Clark where obesity was highly prevalent among our patients, 65.5% of our patients have BMI >30 Kg/m2.

Twenty patients were on insulin and 37 were not using insulin. There was no significant different between the 2 groups in terms of fibrosis score which implies insulin resistance in both categories (resistance to internally produced insulin or exogenously introduced insulin that lead to obesity which complicate NAFLD and increased fibrosis core in both).

Lifestyle modification consisting of diet, exercise, and weight loss has been advocated to treat patients with NAFLD in all guidelines [31-33].

Sedentary behavior can be defined as a state of prolong sitting, laying down, consuming very small amounts of energy in which the muscles are inactive (low-intensity exercises or movement) [34,35].

Sedentary behavior will increase in people who have a metabolic syndrome, excessive adiposity, cardiovascular disease and type 2 diabetes mellitus [36,37]. Sedentary time of NAFLD patient is

nearly half an hour extra than healthy people [36]. We found that 39.9% of subjects in our study have a sitting time more than 7 hours per day which was reflected on their high BMI and dyslipidemia. However this wasn`t shown to be significantly associated with NAFLD. This may be due to the fact that sitting time is not a direct risk factor for NAFLD. On the other hand, sedentary life style leads to obesity and dyslipidemia which in turn lead to prognoses of NAFLD.

The incidence of NAFLD is positively associated with the increase in sitting time independent of physical activity and exercises [38 and 39]. A study done in 2016 in China was reported that the prevalence of NAFLD depends on the sitting time and it will be higher in people with a sitting time of 7.1hours/day and longer [38]. Another study reported that the moderate or intense exercises have significant benefit for NAFLD patients [40]. The reduction in the physical activity have documented especially in NAFLD patient who also suffered from diabetes [41].

Table 1. Socio-demographic characteristics of the NAFLDs patients (n=203)

Variables and its categories		Frequency (n)	Percentage (%)
Residency	City	80	39.4
	Village	121	59.6
	Camp	2	1
Gender	Male	84	41.4
	Female	119	58.6
Age	20-39 years	25	12.3
	40-59 years	102	50.2
	≥ 60 years	76	37.4
	Minimum	20	
	Maximum	87	
	Mean	54.78	
	Standard deviation	12.27	
BMI	18.5-24.4 Kg/m^2	20	9.9
	24.5-30 Kg/m^2	50	24.6
	More than 30 Kg/m^2	133	65.5
Are you smoker	Yes	42	20.7
	No	156	76.8
	Missing	5	2.5
Sitting time	1-3 hours	84	27.6
	4-7 hours	65	32.0
	More than 7 hours	81	39.9
	Missing	1	0.5
Moving time	1-3 hours	76	37.4
	4-7 hours	70	34.5
	More than 7 hours	56	27.6
	Missing	1	0.5
Number of meals	1	20	9.9
	2	111	54.7
	≥ 3	70	34.5
	Missing	2	1

Abbreviation: BMI, body mass index
**significance at α≤0.05*

NAFLD has a close association with obesity, mainly visceral obesity. It is considered as the dominant risk factor for NAFLD occurrence [19,25]. It is major leading cause of cardiovascular diseases [26].

In our study we have found that, 67 patients have dyslipidemia and 62 of them were on statins (atorvastatin). This was not associated with improvement of their overall NAFLD or dyslipidemia, rather they have worse condition comparing to other patients in this study whether they were using statins or not. This was clear as they have high fibrosis score. This might be explained in 2-ways; Patients are hesitant to use statins in our community due high cost, side effects or believes they are nt working for their condition.

Table 2. Diseases account for the development of NAFLD (n=119)

Variables and its categories			**Fibrosis score**			**Total**	**P-value (Sig)**
			more than 0.676	**-1.455_0.676**	**less than -1.455**		
Are you diabetic?	Yes	Count	51	7	0	58	0.000*****
		% within fibrosis score	62.2%	21.2%	0.0%	48.7%	
		% of Total	42.9%	5.9%	0.0%	48.7%	
	No	Count	31	26	4	61	
		% within fibrosis score	37.8%	78.8%	100.0%	51.3%	
		% of Total	26.1%	21.8%	3.4%	51.3%	
Number of years of diabetes	≤ 2 years	Count	13	5	0	18	0.167
		% within fibrosis score	28.9%	71.4%	0.0%	34.6%	
		% of Total	25.0%	9.6%	0.0%	34.6%	
	3-5 years	Count	13	1	0	14	
		% within fibrosis score	28.9%	14.3%	0.0%	26.9%	
		% of Total	25.0%	1.9%	0.0%	26.9%	
	6-10 years	Count	8	0	0	8	
		% within fibrosis score	17.8%	0.0%	0.0%	15.4%	
		% of Total	15.4%	0.0%	0.0%	15.4%	
	10> years	Count	11	1	0	12	
		% within fibrosis score	24.4%	14.3%	0.0%	23.1%	
		% of Total	21.2%	1.9%	0.0%	23.1%	
Do you have hyperlipidemia?	Yes	Count	58	16	2	76	0.023
		% within fibrosis score	71.6%	48.5%	50.0%	64.4%	
		% of Total	49.2%	13.6%	1.7%	64.4%	
	No	Count	23	17	2	42	
		% within fibrosis score	28.4%	51.5%	50.0%	35.6%	
		% of Total	19.5%	14.4%	1.7%	35.6%	
Number of years of	≤ 2 years	Count	20	7	2	29	0.507

Variables and its categories			**Fibrosis score**			**Total**	**P-value (Sig)**
dyslipidemia		% within fibrosis score	40.0%	46.7%	100.0%	43.3%	
		% of Total	29.9%	10.4%	3.0%	43.3%	
	3-5 years	Count	15	5	0	20	
		% within fibrosis score	30.0%	33.3%	0.0%	29.9%	
		% of Total	22.4%	7.5%	0.0%	29.9%	
	6-10 years	Count	13	2	0	15	
		% within fibrosis score	26.0%	13.3%	0.0%	22.4%	
		Table 2 continued.					
		% of Total	19.4%	3.0%	0.0%	22.4%	
	10> years	Count	2	1	0	3	
		% within fibrosis score	4.0%	6.7%	0.0%	4.5%	
		% of Total	3.0%	1.5%	0.0%	4.5%	
Are you hypertensive?	Yes	Count	54	10	0	64	0.000*****
		% within fibrosis score	65.9%	30.3%	0.0%	35.8%	
		% of Total	45.4%	8.4%	0.0%	35.8%	
	No	Count	28	23	4	55	
		% within fibrosis score	43.1%	69.7%	100.0%	46.2%	
		% of Total	23.5%	19.3%	3.4%	46.2%	

**significance at α≤0.05*

Table 3. Role of hypoglycemic, antihypertensive and lipid lowering agents in NAFLD management (n=119)

Variable and its categories			Fibrosis score			P- value (sig)
			More than 0.676	-1.455_0.676	Less than -1.455	
Diabetes mellitus medications						
Do you take Metformin.	Yes	Count	36	5	0	.975
		% within fibrosis score	72.0%	71.4%	0.0%	
		% of Total	63.2%	8.8%	0.0%	
	No	Count	28.0%	28.6%	0	
		% within fibrosis score	24.6%	3.5%	0.0%	
		% of Total	50	7	0.0%	
Do you take Insulin.	Yes	Count	18	2	0	.706
		% within fibrosis score	36.0%	28.6%	0.0%	
		% of Total	31.6%	3.5%	0.0%	
	No	Count	32	5	0	
		% within fibrosis score	64.0%	71.4%	0.0%	
		% of Total	56.1%	8.8%	0.0%	
Do you take Sulfonylurea	Yes	Count	23	2	0	.393
		% within fibrosis score	46.0%	28.6%	0.0%	
		% of Total	40.4%	3.5%	0.0%	
	No	Count	27	5	0	
		% within fibrosis score	54.0%	71.4%	0.0%	
		% of Total	47.4%	8.8%	0.0%	
Hypertension medications						
Do you take Beta blockers	Yes	Count	34	4	1	.413
		% within fibrosis score	60.7%	36.4%	100.0%	
		% of Total	50.0%	5.9%	1.5%	
	No	Count	22	7	0	
		% within fibrosis score	39.3%	63.6%	0.0%	
		% of Total	32.4%	10.3%	0.0%	

Variable and its categories			**Fibrosis score**			**P- value (sig)**
			More than 0.676	**-1.455_0.676**	**Less than -1.455**	
Diabetes mellitus medications						
Do you take ACEIs	Yes	Count	25	3	0	.182
		% within fibrosis score	44.6%	27.3%	0.0%	
		% of Total	36.8%	4.4%	0.0%	
	No	Count	31	8	1	
		% within fibrosis score	55.4%	72.7%	100.0%	
		% of Total	45.6%	11.8%	1.5%	
		Table 3 continued				
Do you take CCBs	Yes	Count	22	3	0	.304
		% within fibrosis score	39.3%	27.3%	0.0%	
		% of Total	32.4%	4.4%	0.0%	
	No	Count	34	8	1	
		% within fibrosis score	60.7%	72.7%	100.0%	
		% of Total	50.0%	11.8%	1.5%	
Dyslipidemia medications						
Do you take Atorvastatin	Yes	Count	51	10	1	.015*
		% within fibrosis score	86.4%	62.5%	50.0%	
	No	Count	8	6	1	
		% within fibrosis score	13.6%	37.5%	50.0%	

Abbreviations: ACEIs, angiotensin-converting-enzyme inhibitors; CCBs, Calcium channel blockers.
**significance at α≤0.05*

On the other hand, we found that, patients in this study use statins at a late stage of their dyslipidemia or atherosclerosis. They were not using stains for reasons or doses related to NAFLD neither did they use them for sufficient time in order to be able to judge on its their efficacy.

Dyslipidemia is a critical comorbidity that is observed in NAFLD patients [65]. It is atherogenic in nature [64]. It is characterized by high triglyceride (TG) and LDL levels and low HDL levels [67]. This atherogenic abnormality increases risk of cardiovascular diseases [68]. The specific mechanism in which hyperlipidemia increases the risk of NAFLD is unclear. It may be associated with increased accumulation of lipids in liver cells [69] as a result of lipid metabolism abnormalities such as increased lipogenesis, ingestion of fatty foods and increase synthesis of very low density lipoprotein (VLDL) in addition to decreased oxidation of free fatty acid [70].

Our study was first study to predict the relationship between hypertension and NAFLD. One common factor for three conditions (dyslipidemia, hypertension and NAFLD) is obesity and or/ increased level of lipids in the body. Atherosclerosis is a major risk factor for hypertension among patients in our study. Hence come the indirect relationship between NAFLD and dyslipidemia that lead to atherosclerosis which lead to hypertension.

Our results came even more controversial where patient who took statins had worse score of NAFLD fibrosis comparing to those who didn't take it, as explained above.

5. CONCLUSION

Tight control of hypertension and dyslipidemia are mandatory among all NAFLD patients. Lifestyle modifications such as low fat and carb diets and exercise, are important measurement to fight obesity, hypertension and diabetes hence they will improve fibrosis in NAFLD patients. studies about use of stains, their dose, timing and adherence are mandatory to judge on statins benefit for NAFLD.

CONSENT AND ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s). This was a multistage process where we have to take permission from the IRC of our University to run the research. Then we took the permission of ministry of health in Ramallah and the district health office in Hebron to run the study in their hospital and to collect data from patients.

We prepared a consent form where we have to take consent from each patient or his guardian to participate in the research and to share information with us for the purpose of research only. We guarantee the confidentiality of his/her personal information. Participants and/or their care giver, or guardian had to sign the consent form on top of the interview-based questionnaire.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 20
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Determination of Early T-cell Precursor Acute Lymphoblastic Leukemia- A Neoplasm with Dual Lineage Phenotype

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ABSTRACT

Early T-cell Precursor Acute Lymphoblastic Leukemia (ETP-ALL) is a subtype of T-ALL/LBL which is derived from thymic cells at the early T-cell precursor (ETP) differentiation stage that have the potential to differentiate into multiple lineages, including lymphoid and myeloid. ETP-ALL accounts for 15% of childhood T-ALL and 10-30% of adult T-ALL. It has characteristic immunophenotypic expression of CD7, a lack of CD1a and CD8, weak expression of CD5 (with <75% positive blasts), and positive expression of one or more stem cell or myeloid markers including CD117, HLADR, CD13, CD33, CD11b, or CD65.ETP-ALL is identified by a well defined gene expression signature including DNMT3A and FAT3 mutations. We here in report two cases of ETP-ALL with brief review of literature.

Keywords: ETP-ALL; T-ALL; CD1a; CD8.

1. INTRODUCTION

T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) is a malignant neoplasm of immature T cell with subtypes that correspond to different T-cell maturation stages [1-4]. These account for 10–15% of childhood and 10-30% of adult ALL cases [5].

Early T-cell Precursor Acute Lymphoblastic Leukemia (ETP-ALL) is a subtype of T-ALL/LBL derived from thymic cells at the early T-cell precursor (ETP) differentiation stage that have the potential to differentiate into multiple lineages, including lymphoid and myeloid [6,7]. World Health Organization (WHO) classification of acute leukemia, 2016 update has included ETP-ALL as a new provisional entity which was kept under early-T-ALL/LBL category in 2008 WHO classification [8].

ETP-ALL accounts for 15% of childhood T-ALL and 10-30% of adult T-ALL [6-11].

It was defined by a distinct immunophenotypic expression of CD7, a lack of CD1a and CD8, weak expression of CD5 (with <75% positive blasts), and positive expression of one or more stem cell or myeloid markers including CD117, HLADR, CD13, CD33, CD11b, or CD65 [6].

ETP-ALL/LBL is also characterized by a distinct molecular profile with a lower frequency of NOTCH1 mutations and frequent occurence of FLT3 and DNMT3A mutations [12-14]. Most importantly, it has comparatively worse outcome in children and young adults than other T-ALL/LBL subtypes [6,15].

The purpose of this case study is to highlight the clinical, immunophenotypic and molecular characteristics of ETP-ALL and discuss two cases experienced at our institution.

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2. CASE REPORT

We have reported total of 12 cases of T-ALL on flowcytometry from July, 2019 to January, 2021 out of which 2 cases are of Adult ETP-ALL(16%).

Case 1: A 35 year old woman presented to OPD with complaints of generalized lymphadenopathy, fever and weakness for 1 months. There was no significant family or past history. On examination, she had pallor, with multiple small, painless nodes palpable in b/l cervical, axillary and inguinal region. Moderate splenomegaly was also present. Respiratory, cardiovascular and neurological examinations were unremarkable. Complete blood count showed moderate anemia (Hb-6.5 g/dl), high TLC (90,000/ul) and mild thrombocytopenia (Platelet count-75,000//ul). Peripheral blood smear confirmed leucocytosis with approximately 82% blasts (Fig. 1). These blasts were small to medium sized with high N:C ratio, round nucleus with irregular contour, condensed chromatin, inconspicuous to prominent nucleoli and scant agranular basophilic cytoplasm (Fig. 2). A provisional diagnosis of MPO negative acute leukemia was made.

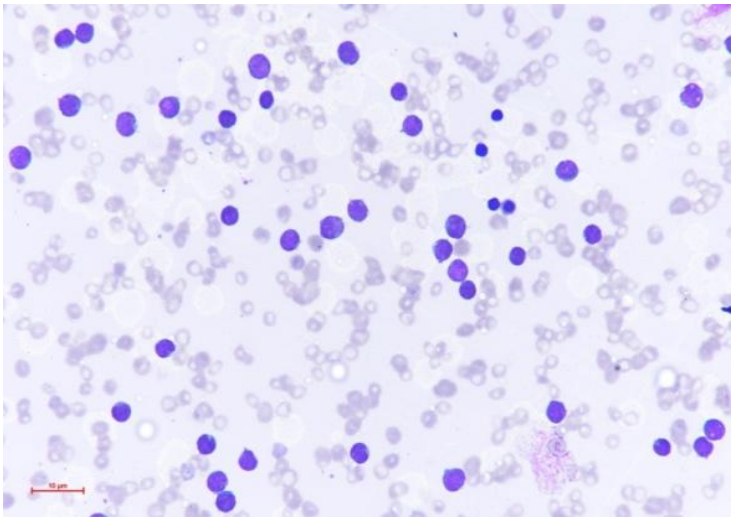


Fig. 1. Leucocytosis with approximately 82% blasts (400x)

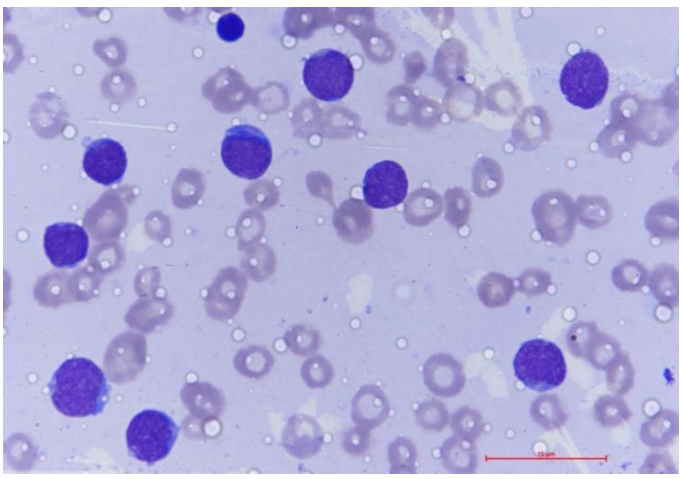
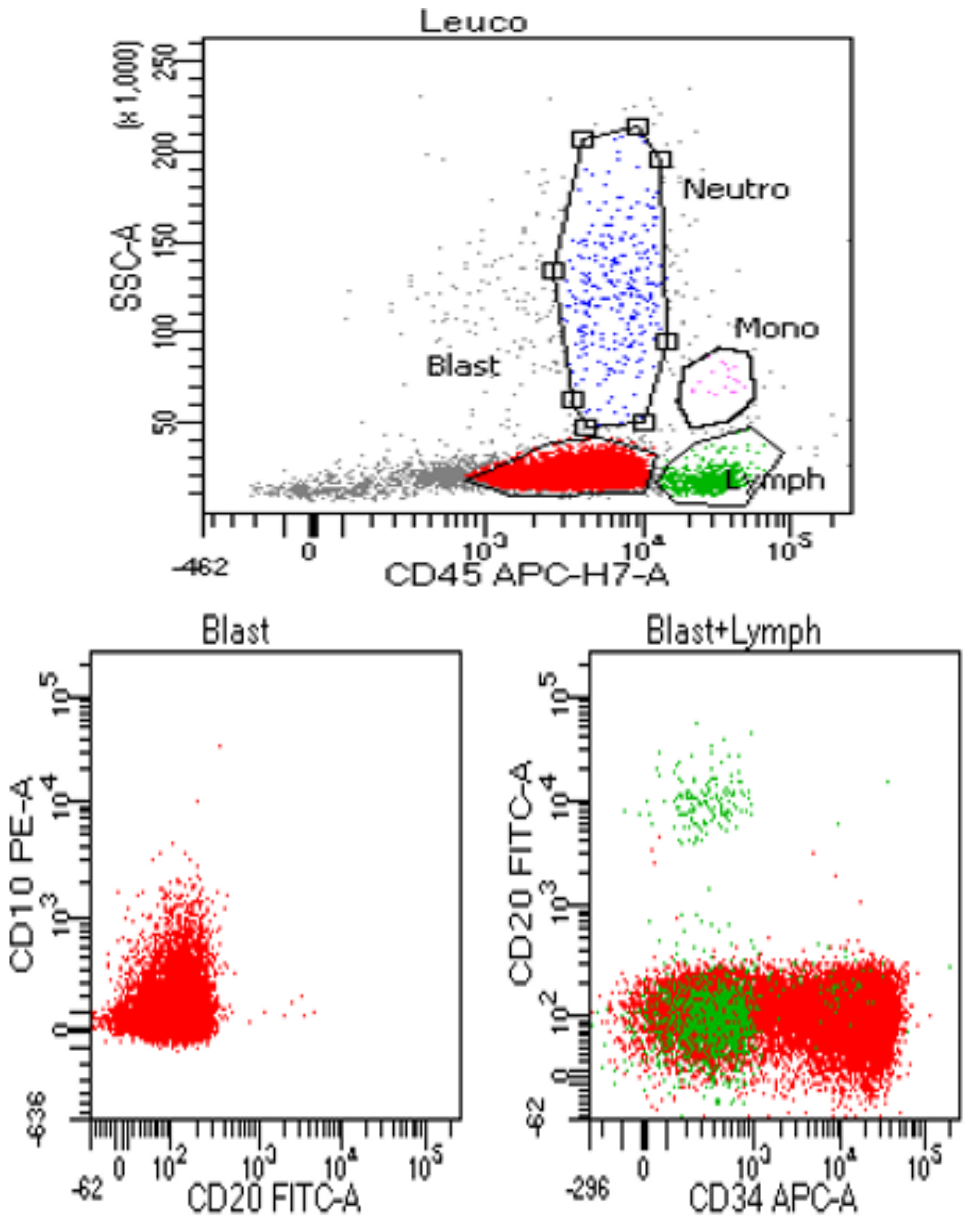


Fig. 2. Small to medium sized blasts with high N:C ratio, round to oval nucleus with irregular nuclear contour, inconspicuous to prominent nucleoli and scanty to moderate agranular basophilc cytoplasm. (1000x)



Population	#Events	%Parent	%Total
All Events	30,000	####	100.0
Singlets	26,751	89.2	89.2
Leuco	26,642	99.6	88.8
Lymph	1,385	5.2	4.6
Mono	43	0.2	0.1
Neutro	592	2.2	2.0
Blast	21,853	82.0	72.8

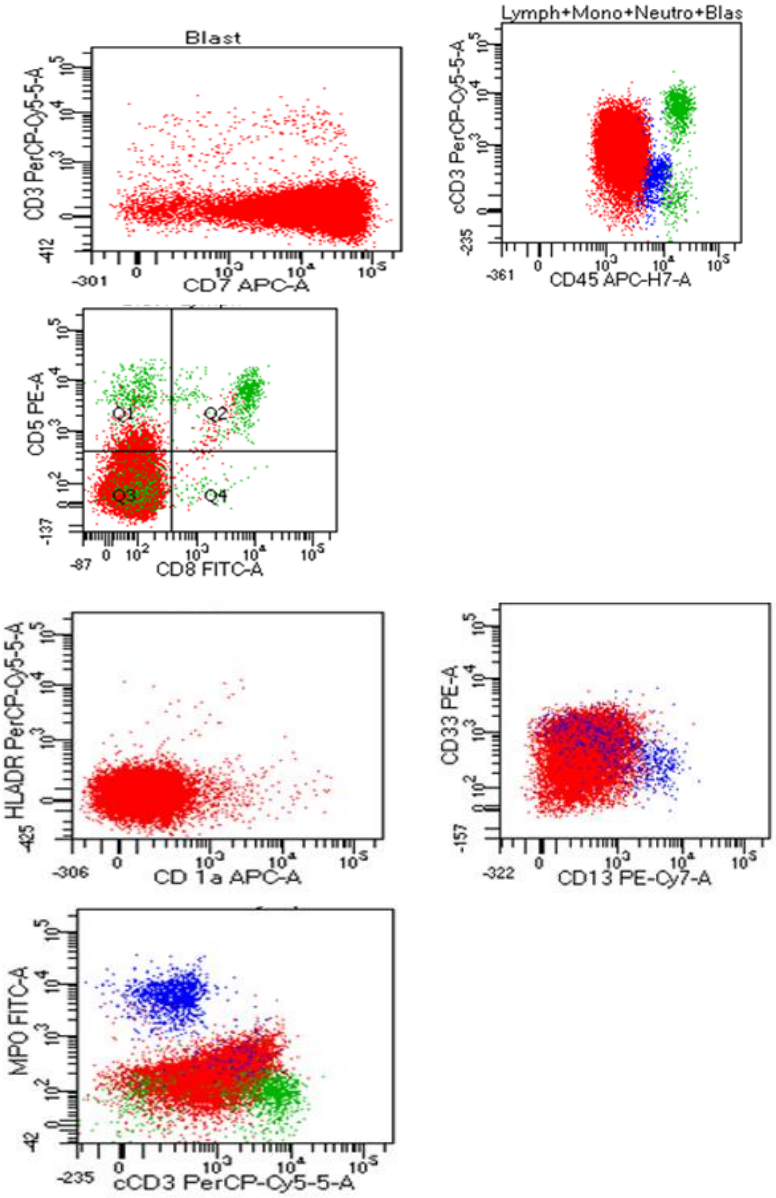


Fig. 3. Scatter plot of flowcytometric analysis of ETP-ALL showing its characteristic immunophenotypic profile

Immunophenotyping of the blasts in the peripheral blood was performed by standard flow cytometry methods, using standardised antibody panels. Immunophenotyping was performed by a FACS Canto II flow cytometer with six-colour reagent panels. It showed 82% cells gated in low side scatter and moderate CD 45 region which was positive for cytoplasmic CD3, CD7, CD5 (dim and partial), CD34, CD13 & CD33 (dim and partial) and negative for surface CD3, CD1a, CD8, HLA-DR and cyto MPO. Final diagnosis of ETP-ALL was made.

Case 2: A 20 yr old male presented to OPD with complaints of generalized lymphadenopathy and fever for 3 months. There was no significant family history or past history. General examination showed pallor with multiple small, painless palpable nodes in b/l cervical and axillary regions. Respiratory, cardiovascular, abdominal and neurological examination was unremarkable. Initial laboratory investigations revealed pancytopenia with severe anemia (Hb-3 g/dl), Moderate leucopenia (TLC-1100/ul) and moderate thrombocytopenia (Platelet count-50,000/ul). Peripheral blood smear

showed 65% atypical cells, confirmed on bone marrow examination which revealed cellular marrow with marked infiltration by blasts. These were small to medium sized with high N:C ratio, round nucleus with irregular contour, condensed to fine granular chromatin, prominent nucleoli and scant to moderate agranular basophilic cytoplasm. Provisional diagnosis of precursor lymphoblastic leukemia was made.

Immunophenotyping of the blasts in the bone marrow sample was performed and it showed 75% cells gated in low side scatter and dim to moderate CD45 region which was positive for cytoplasmic CD3, CD7, CD5 (dim and partial), CD34, CD38, HLA-DR, CD33 (dim and partial) and CD117 (dim and partial) and negative for surface CD3, CD1a, CD8, CD13 and cyto MPO. Final diagnosis of ETP-ALL was given.

3. DISCUSSION

ETP-ALL is a recently described subgroup of T-ALL distinguished by very early arrest in T-cell differentiation, identified by a well defined gene expression signature and immunophenotype [6,9,16].

Normal early T-cell precursors (ETPs) are a subtype of thymocytes, which have migrated from the bone marrow to the thymus, and retain multilineage differentiation potential, indicating that they are derived from hematopoietic stem cells [17-19]. However, recently, a mouse model of T-ALL using a Sleeping-Beauty-based transposon system suggested that ETP-ALL may be derived from more mature T-cells [20]. Thus, the exact cellular origin of ETP-ALL is not clear.

Recently, a study attempted to make diagnosis of ETP-ALL using the expression of CD5 and concluded that CD5- negative T-ALL could be diagnosed as ETP-ALL because CD5 negativity was associated with positive myeloid/stem cell antigens but not CD1a and CD8 expressions [21].

Currently, precise immunophenotyping is the most important tool to make a diagnosis of ETPALL, distinguishing ETP-ALL from classical T-ALL. In our case report both cases were diagnosed by flowcytometric analysis.

Both pediatric and adult ETP-ALL show distinct mutations. Pediatric ETP-ALL has a higher expression of oncogenic transcription factors: LMO1, LMO2, LYL1, and ERG [6,7]. Whole-genome sequencing studies showed that ETP-ALL has a high frequency of activating mutations in the genes involved in cytokine receptor and RAS signaling (e.g., NRAS, KRAS, FLT3, IL-7R, JAK3, LAK1, SH2B3, and BRAF) and inactivating mutations in the genes encoding key transcription factors involved in hematopoietic development (e.g., GATA3, ETV6, RUNX1, IKZF1, and EP300) and involved in epigenetic gene control (e.g., EZH2, EED, SUZ12, SETD2, and EP300 genes) [7]. In adult ETP-ALL patients whole exome sequencing revealed a distinct mutation spectrum from that of pediatric ETP-ALL, particularly in affecting genes involved in epigenetic regulation with higher frequencies of DNMT3A and FAT3 mutations [14]. *DNMT3A* alterations in lymphoid malignancies are limited to T-lineage disease. In all cases, *DNMT3A* mutations increase in frequency with age, and are extremely rare in children and adolescents [22].

Collectively, characteristic immunophenotype and distinct genetic profiles distinguish ETP-ALL from classical T-ALL. The characteristic gene profile of ETP-ALL may provide new therapeutic strategies for this leukemia.

Coustan-Smith et al. reported that patients with ETP-ALL showed a poor initial response to standard intensive chemotherapies and unfavorable outcomes [6]. Recent protocols for T-ALL patients include consecutive phases of induction, consolidation, delayed intensification, and maintenance, with drug combinations that commonly include doxorubicin or daunorubicin, dexamethasone or prednisone, vincristine, asparaginase, cyclophosphamide and cytarabine, together with methotrexate and intrathecal chemotherapy as prophylaxis for CNS infiltration. In past, ETP-ALL has been associated with poor prognosis. but in recent years application of early response-based intensification regimens has greatly improved the outcome of these patients [23].

In the TLLSGL99-15 study, three of four relapsed ETP-ALL patients were successfully treated with allogenic hematopoietic stem cell transplantation (allo-SCT), indicating that allo-SCT could be an effective therapeutic option for ETP-ALL, hence mandating the precise diagnosis [15].

4. CONCLUSION

Diagnosis of ETP-ALL needs combination of cell morphology, cytochemical staining as well as flowcytometric analysis. The main purpose of this report is to make physicians aware of this entity, its controversial prognostic significance and the need for novel treatment strategies.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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