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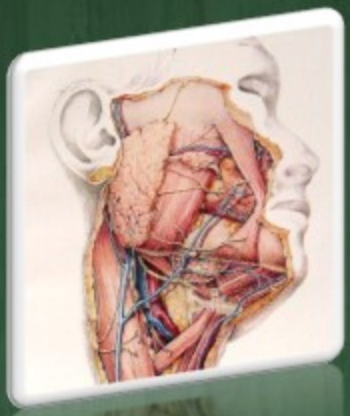
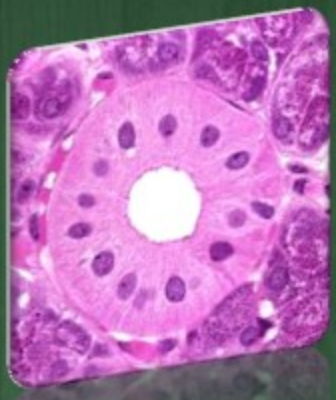

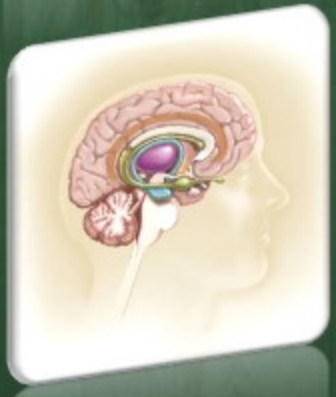

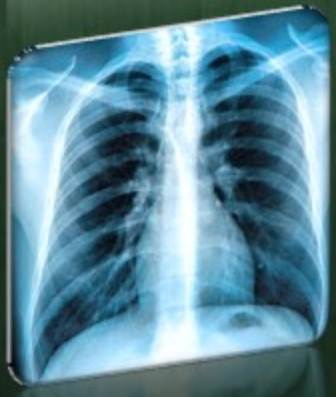

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Original Article

IN VITRO ANTI-PROLIFERATIVE ACTIVITY OF FUCOXANTHIN ON HeLa CELLS

Alok Saxena[1], Anupama Mahajan[1], Suryakant Nagtilak[2], S.N. Bahuguna[3]

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ABSTRACT

Introduction: A couple of years ago, cancer was a prime cause of death and ranked 6th in developed countries. Cancer is a genetic disorder occurring due to mutation in DNA. Carotenoids such as fucoxanthin, lutein, zeaxanthin, lycopene, and others are natural phytopigments which reportedly confer several benefits to human health. Fucoxanthin, a major marine carotenoid, is present in seaweeds such as "*Hijikia fusiformis*, *Undaria pinnatifida*, and *Sargassum fulvellum*". It possesses antitumor and anti-inflammatory activity by inducing apoptosis and reducing free radicals. Owing to the prior suggestion of the anti-proliferative potential of fucoxanthin in various cancers, the present study was performed to investigate its in-vitro anticancer properties on the cervical cancer cell line (HeLa) against a standard drug-tamoxifen.

Materials and methods: Cultured HeLa cells were treated with nine different concentrations each of fucoxanthin and tamoxifen for 24 and 48 hrs respectively. MTT assay was performed to measure cell viability. Anti-proliferative potency against cancer cells was evaluated by IC50 values. Furthermore, annexin-V/FITC-PI staining coupled with flow-cytometry was used to mechanistically evaluate the concentration-dependent (three concentrations) apoptosis induced by the drugs.

Results: Fucoxanthin was found capable of inhibiting cell proliferation and inducing apoptosis in HeLa cells.

Conclusions: Results suggest that the in-vitro anticancer effects of fucoxanthin against cervical cancer cells (HeLa) are comparable to tamoxifen.

Keywords: Looping, Internal carotid artery, Variations

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INTRODUCTION

A recent report from the World Health Organization claimed that cervical cancer stepped up to 4th rank among cancers in women.[1] Chemotherapy is the most extensive method for cancer treatment but its side effects and multidrug resistance are greater challenges.[2] The carotenoids have gained much attention due to their antioxidant properties and have played a therapeutic role in cancer suppression.[3] Fucoxanthin (Fx), an orange color pigment of edible brown algae possesses antitumor activity, induces apoptosis, anti-inflammatory effects, and radical scavenging activity.[4,5] A very few researches based on cellular model revealing anticancer effects of Fx have been published. Based on previous evidences, an in-vitro study was designed to investigate the anticancer properties of Fx on HeLa cells against a standard anticancer drug-Tamoxifen (Tx).

MATERIALS AND METHODS

1. Procurement of testing materials

Fucoxanthin and Tx,[6] (purity 98%, analytical grade) were procured from Sigma Aldrich, India. Cervical cancer cell lines (HeLa) were obtained from the National Centre for Cell Science (NCCS), Pune.

2. Cell culture

HeLa cells were grown in 5 ml Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS and 1% penicillin-streptomycin. Cells were maintained at 37°C in a humidified atmosphere of 5% CO2 using a CO2 incubator. Once cells were 95% confluent, they were harvested by trypsinization process. Thereafter, the cells were pelleted using centrifugation at 1000 rpm for 5min. The supernatant was discarded and the cells were resuspended in fresh assay media (2ml) to achieve 1million cells/ml. Cells were then pipetted into 96 well plates. Ten thousand cells were added in 100 µl medium/ well a day before performing the assay. The cell suspensions were incubated for 24 hrs at 37°C to provide cell adherence. After 24 hrs, cells were inoculated with 100 µl of each concentration of serially diluted drug (Fx and Tx) preparation. Cancer cells were treated with 9 different concentrations (20, 10, 5, 2.5, 1.25, 0.625, 0.3125 and 0.078 µM) of Fx, and Tx in triplicates for 24 and 48 hrs.

3. Cell proliferation assay

MTT Assay:

MTT (3-4,5-dimethythiazol 2-yl-2,5-diphenyl tetrazolium bromide) assay was used to quantify the cytotoxic effect of Fx, and Tx on HeLa cells. A total of 5mg MTT reagent was prepared in 1ml of PBS. Thereby, 20 µl MTT was added to all the wells and the plate was incubated for 4 hrs at 37°C. After the incubation period, the assay media with MTT reagent was discarded and each well was fed with 100µl of dimethylsulfoxide to dissolve the formazon crystals formed from the reaction. Further, a 96-well plate was incubated in dark at room temperature for one hr. Absorbance was read at 570 nm with a reference wavelength of 650 nm using a microplate reader (Bio-Rad Lab, Model 3550). The effects of Fx and Tx on percent viability of HeLa cells were tested.

4. Determination of half maximal inhibitory concentration (IC50)

The absorbance (measured in MTT assay) was used as a proxy for cell viability. The data set from each cell-line type and drug treatment pair were fitted with a sigmoid curve, separately at 24 and 48 hrs time points. Consequently, IC50 estimates were obtained from each dataset which was then used to make inference about their anti-proliferative potencies.

5. Measurement of apoptosis using Annexin V-FITC/PI staining

FITC annexin V apoptosis detection kit was procured from BD Pharmingen, India and the recommended protocol was followed. Cancer cells were seeded in 6-well plates and treated with 20, 10, and 5 μM (in triplicates) concentration of each of Fx, and Tx for 24 hr. After that, cells were washed with cold PBS twice and suspended in 100 μl 1X binding buffer. 100 μL solution of cell suspension (5x 104 cells) + 5 μL FITC annexin V + 5 μL PI was added in each well and labelled as: one well each for 3 concentrations of 3 drugs; control, FITC, PI, FITC+PI and incubated for 15 min. at room temperature in the dark. Thereafter, 400 μl of 1x binding buffer was added in each well to analyze the reading within one hour using flow cytometry (BD Accuri C6).

Statistical Analysis

A two-way grouping between analysis of variance (ANOVA) was applied to evaluate the effect of time points and treatment levels as well as the effect of treatment types (Fx, Tx) and treatment levels on % viability of HeLa cells. Bonferroni adjusted simple main effects analyses were carried out to evaluate the difference between two levels of time points and drug types (at each level of treatment. Further, Tukey HSD post hoc analyses were done separately at respective time points as well as drug types. For all purposes $p>0.05$ was considered to indicate statistical significance. All the values of MTT assay were analysed using SPSS version 21. The potency of carotenoids was evaluated by determining IC50 values using Graph Pad Prism 8 software. Kruskal Wallis one way ANOVA was done to measure the apoptosis of three different doses of Fx, and Tx each. All values of Flow cytometry to quantify the different stages of cell death were analysed by MATLAB R2015a software.

RESULTS

1. Cell Proliferation Assay

1.1. Effect of Fx on HeLa cells

There was a statistically significant interaction between the factors of time points and treatment levels on the effect of % viability, $F (9, 40) = 3.42$, $p = 0.0033$, partial $\eta 2= 0.435$. The mean differences of % viability between 24 hrs and 48 hrs were significant at 10 μM ($p = 0.014$), 5 μM ($p = 0.033$), 2.5 μM ($p < 0.01$), 1.25 μM ($p = 0.004$), 0.625 μM ($p = 0.014$), 0.3125 μM ($p = 0.002$), 0.15625 μM ($p = 0.003$), 0.078 μM ($p < 0.01$) respectively. Further, Tukey HSD post hoc done separately at 24 and 48 hrs time points showed that differences between control and Fx (20 μM) were both significant ($p < 0.01$). (Fig: 1)

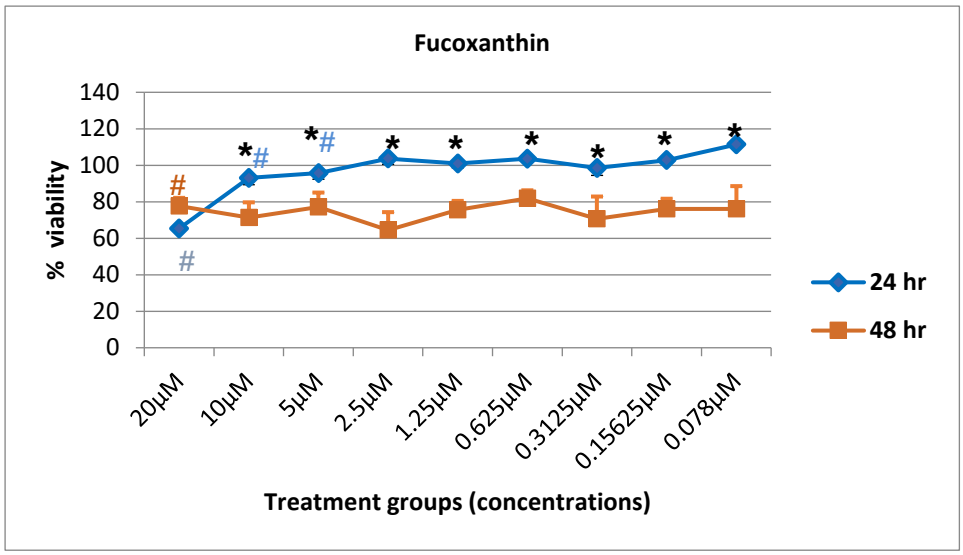

Fig. 1. * significant difference b/w 24 and 48 h
significant difference b/w 24 h and control
significant difference b/w 48 h and control

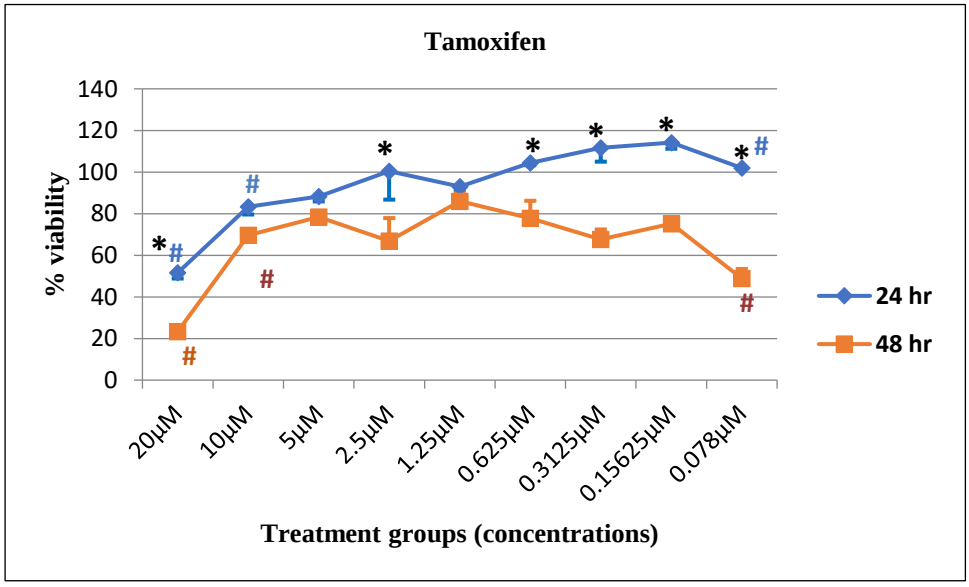

Fig. 2. * Significant difference b/w 24 and 48 h
Significant difference b/w 24 h and control
Significant difference b/w 48 h and control

1.2. Effect of Tx on HeLa cells

There was a statistically significant interaction between the factors of time points and treatment levels on % viability, F (9, 40) = 5.37, p < 0.01, partial η2 = 0.55. The mean differences of % viability between 24 hrs and 48 hrs were significant at 20 μM (p = 0.001), 2.5 μM (p < 0.01), 0.625 μM (p = 0.001), 0.3125 μM (p< 0.01), 0.15625 μM (p < 0.01), 0.078 μM (p < 0.01) respectively. Tukey HSD post hoc showed after 24 hrs the mean differences of % viability was significant between control and Tx concentrations at 20 μM (p < 0.01), 10 μM (p = 0.003) and 0.078 μM (p = 0.002) respectively. Likewise, after 48 hrs the differences between control and Tx groups were significant at 20 μM (p < 0.01), 10 μM (p = 0.003) and 0.078 μM (p = 0.001) respectively (Fig: 2).

1.3. Comparative effect of Fx and Tx on HeLa cells at 24 hrs

A statistically significant interaction was found between the factors of treatment types and treatment levels on % viability, F (9, 40) = 2.434, p= 0.026, partial η2= 0.35. Bonferrroni adjusted simple main effects analyses revealed the mean differences of % viability between Fx and Tx treatments were significant at 20 μM (p = 0.022) and 0.3125 μM (p = 0.029) respectively (Fig: 3).

1.4. Comparative effect of Fx and Tx on HeLa cells at 48 hrs:

A statistically significant interaction was found between the factors of treatment types and treatment levels on % viability, F (9, 40) = 3.84, p= 0.001, partial η2= 0.46. Bonferroni adjusted simple main effects analyses revealed that the mean differences of % viability between Fx and Tx treatments were significant at 20 μM (p < 0.01) and 0.078 μM (p= 0.008). This suggests that after 48 hr incubation Fx was largely comparable in effect as Tx in controlling the proliferation of HeLa cells in vitro (Fig: 4).

2. Potency of carotenoids

With HeLa cells, Fx and Tx were more potent (lower IC50) after 24 hr incubation (Fx IC50 = 7.06μM, Fig.5a; Tx IC50 = 5.63 μM, Fig.6a), than their respective 48 hrs values (IC50 = 226.2 μM, Fig.5b; IC50 = 8.85μM, Fig.6b).

3. Apoptosis Assay

3.1 Treatment of HeLa cells with Fx:

After treating HeLa cells with three different concentrations (in triplicates) of Fx for 24 hr, the distribution of cells in various stages of cell death were as follows: (Fig: 7, 7a, 7b, 7c).

Lower left (LL) Quadrant (Unstained cells): After treatment with Fx (F1= 20μM, F2= 10μM, and F3= 5μM) the distribution of cells was 80.44±0.85%, 53±0.56% and 44±0.46% respectively.

Upper left (UL) Quadrant (Early apoptosis): After treatment with Fx (F1= 20μM, F2= 10μM and F3= 5μM) the distribution of cells was 12.4±0.13%, 23.6±0.25% and 32.3±0.34% respectively.

Upper right (UR) Quadrant (Late apoptosis): After treatment with Fx (F1= 20μM, F2= 10μM and F3= 5μM) the distribution of cells was 7.25±0.08%, 23.6±0.25% and 23.1±0.24% respectively.

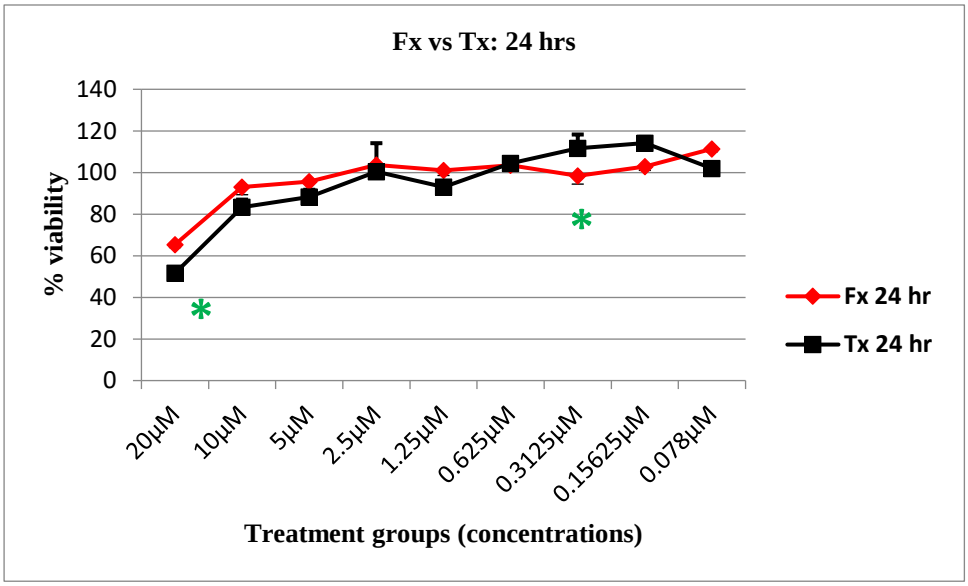

Fig. 3. * **Comparison between Fx and Tx at 24 hr, P<0.05**

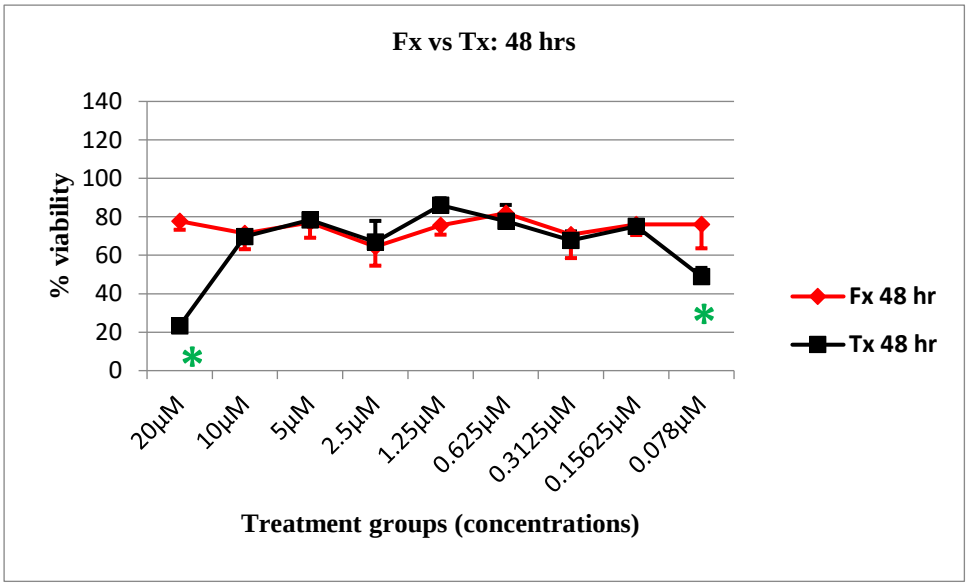

Fig. 4. * **Comparison between Fx and Tx at 48 hr, P<0.05**

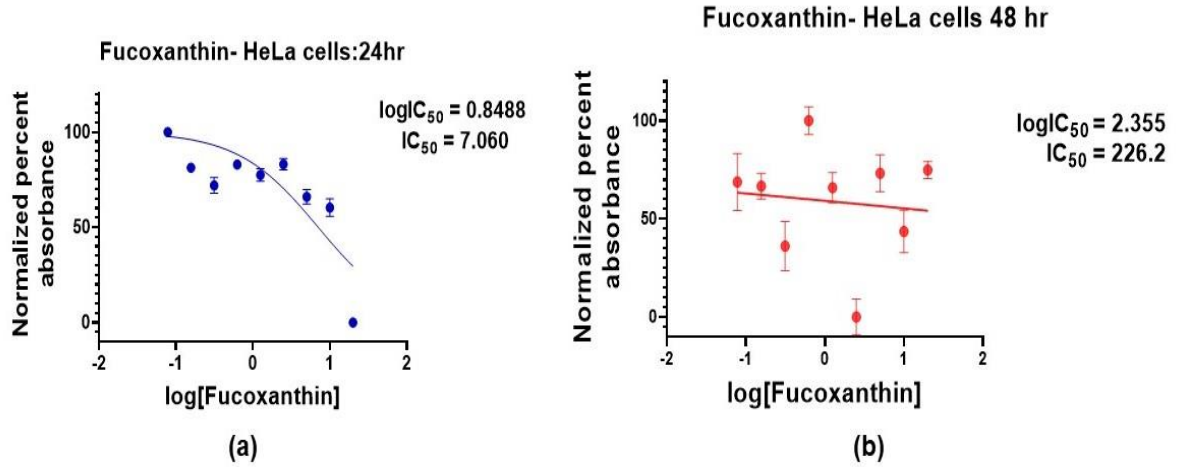

Fig. 5. The potency of Fx on HeLa cells

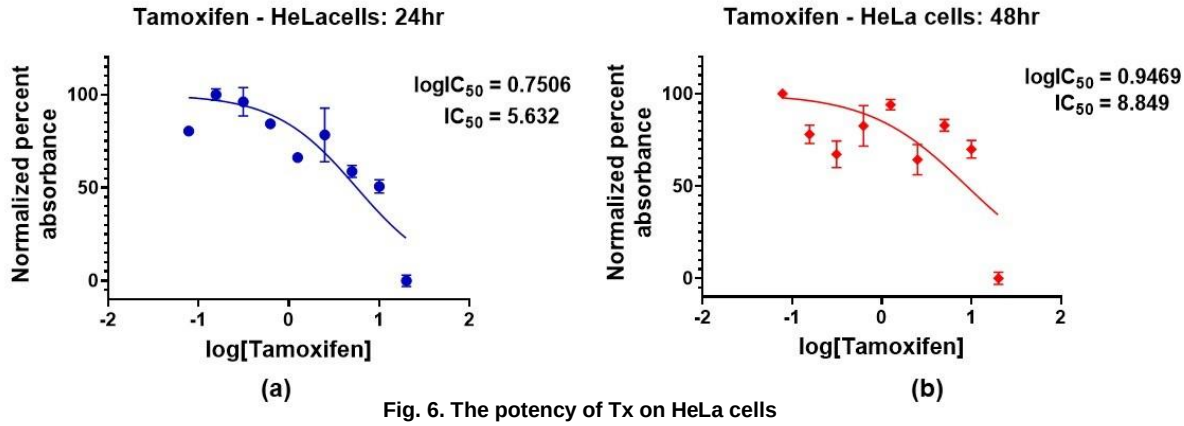

Fig. 6. The potency of Tx on HeLa cells

Lower right (LR) Quadrant (necrosis): After treatment with Fx (F1= 20µM, F2= 10µM and F3= 5µM) the distribution of cells was 0.33±0.003%, 0.45±0.004% and 1.31±0.01% respectively.

3.2 Treatment of HeLa cells with Tx:

After treating HeLa cells with three different concentrations (in triplicates) of Tx, the distribution of cells in various stages of cell death was as follows: (Fig: 8. 8a, 8b, 8c)

Lower left (LL) Quadrant (Unstained cells): After treatment with Tx (T1= 20µM, T2= 10µM, and T3= 5µM) the distribution of cells was 51±0.53%, 54.7±0.6%, and 27.6±0.29% respectively.

Upper left (UL) Quadrant (Early apoptosis): After treatment with Tx (T1= 20µM, T2= 10µM, and T3= 5µM) the distribution of cells was 15.34±0.16%, 35.65±0.38%, and 44.6±0.47% respectively.

Upper right (UR) Quadrant (Late apoptosis): After treatment with Tx (T1= 20µM, T2= 10µM, and T3= 5µM) the distribution of cells was 34.02±0.36%, 7.22±0.08%, and 27.11±0.28% respectively.

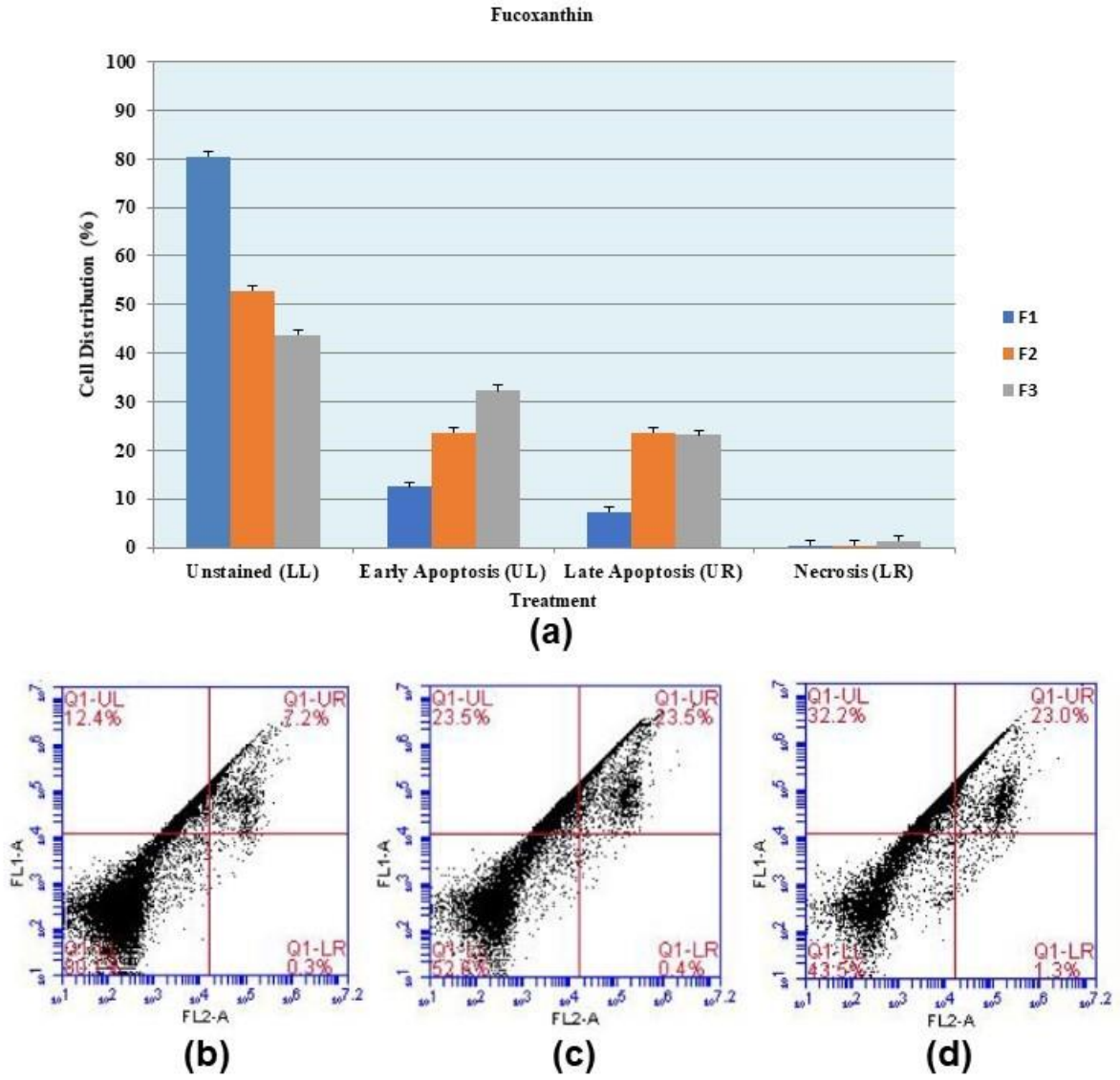

Fig. 7. (a) Distribution of Fx treated HeLa cells (in triplicates) in various stages of apoptosis. Flow cytogram representing percentages of cells in various stages: LL -unstained cells, UL- early apoptosis, UR- late apoptosis, LR- necrosis. HeLa cells were treated with Fx (in triplicates); F1= 20µM (Fig. 7a), F2= 10µM (Fig. 7b), and F3= 5µM (Fig. 7c); Annexin V-FITC/PI staining

Lower right (LR) Quadrant (necrosis): After treatment with Tx (T1= 20µM, T2= 10µM, and T3= 5µM) the distribution of cells was 0.08±0.0008%, 2.85±0.03%, and 1.18±0.01% respectively.

Upon Post Hoc comparison, F1 of late apoptotic cells was significantly lesser as compared to T1 (p=0.0198); and F1 of necrotic cells was significantly lesser than T2 (p=0.0327). Upon Post Hoc comparison T2 of unstained cells was significantly greater as compared to T3 (p=0.0384); T1 of late apoptotic cells was significantly higher as compared to T2 (p=0.0116), and T2 of necrotic cells was significantly higher than T3 (p=0.0066). All other concentrations were statistically insignificant.

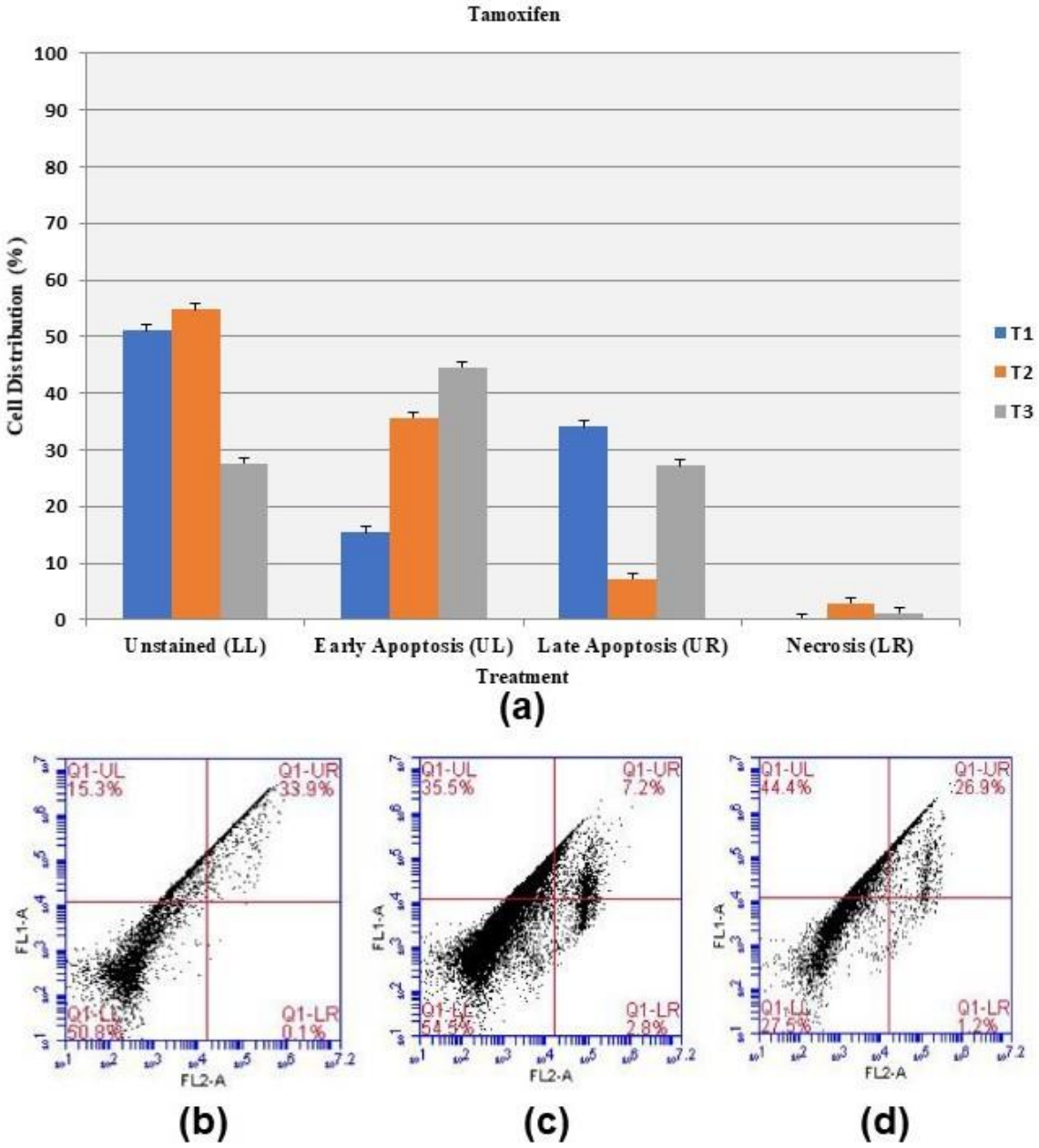

Fig. 8. (a) Distribution of Tx treated HeLa cells (in triplicates) in various stages of apoptosis Flow cytogram representing percentages of cells in various stages: LL -unstained cells, UL- early apoptosis, UR- late apoptosis, LR- necrosis. HeLa cells were treated with Tx (in triplicates); T1= 20µM (Fig. 8a), T2= 10µM (Fig. 8b), and T3= 5µM (Fig. 8c); Annexin V-FITC/PI staining

DISCUSSION

Cervical cancer CasKi cells were treated with Fx alone and in combination with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). A combination of Fx + TRAIL resulted in increased cytotoxicity against cancer cells. [7] The anticancer potential of Fx and TRAIL was evaluated on human cervical cancer SiHa, HeLa, and Caski cell lines. TRAIL treated SiHa, HeLa, and Caski cell lines resulted in reduced cell viability in a dose-dependent manner. SiHa cells treated with 100 ng/mL TRAIL inhibited the cancer cells growth by 14.6% whereas 5ng/Ml concentration of

TRAIL significantly reduced cell viability by 63.3% and 68.5% in CaSki and HeLa cells. SiHa cells were treated with 0.5 μM/L Fx, 100ng/mL TRAIL and 0.5 μM/L Fx+ TRAIL for 48 hrs to measure apoptosis. Flow cytometry revealed that the synergistic effect of combined therapy of Fx and TRAIL increased the number of apoptotic cells by 3.6 fold as compared to the treatment with Fx alone and TRAIL alone in SiHa cells by suppressing p13k/AKT/NF-κB pathway. It was also suggested that TRAIL-induced cell death in cancer cells and acted as an anticancer agent in the treatment of cervical cancer. [8]

In the present study, HeLa cells were treated with three different concentrations (in triplicates) of Fx: 20 μM, 10 μM and 5 μM for 24 hrs to measure apoptosis in early and late stages (Fig, 7a, 7b, 7c). After treatment with 20 μM, 10 μM and 5 μM of Fx the distribution of apoptotic cells was 12.4±0.13%, 23.6±0.25% and 32.3±0.34% respectively in early apoptotic stage and 7.25±0.08%, 23.6±0.25% and 23.1±0.24% respectively in a late apoptotic stage. (Fig.7). The findings of Jin et al [8] did not expose much information about anticancer effects of Fx on HeLa cells but in present experiment Fx arrested HeLa cells growth and induced apoptosis.

In a previous study, autophagy was detected in HeLa cells induced by Fx. An imbalance between the formation and expulsion of autophagosomes may alter the efficient autophagy. Cell proliferation and cell death was detected using MTT assay and Annexin V-FITC/PI staining respectively. HeLa cells were treated with a dose of 10-80 μM Fx that significantly increased the cytotoxicity in a dose-dependent manner at 48 hrs post-incubation with an IC50 value of 55.1±7.6 μM. Cell cycle arrest at the G0/G1 Phase was mediated by the inhibition of the AKT signalling pathway. The regulatory proteins of the cell cycle were also controlled by Fx by upregulation of p21 expression and downregulation CDK2 and cyclin D1 expression. Fucoxanthin did not induce cell death in the early apoptosis stage after 48 and 72 hrs. These findings suggested that Fx persuaded HeLa cells toxicity by increasing the expression of autophagy mediators Beclin 1 and LC3 II and by inhibiting AKT/mToR pathway.[9]

Cervical cancer cells were treated with different concentrations of Fx (0.1μM/L-25μM/L) for 24 and 48 hrs. The MTT assay revealed the decreased cell viability with the treatment of Fx in a dose-dependent manner at 24 hrs. Annexin V-FITC/PI staining revealed that treatment of 0.5μM Fx increased the number of apoptotic cells to 53.4% in the early apoptotic stage after 48 hrs.[10]

In the present experiment, HeLa cells were treated with nine different concentrations (20 μM to 0.078 μM) of Fx for 24 and 48hrs. Fucoxanthin alone showed better results at 20μM at 24 hrs. where it showed less % viability as compared to 48 hrs. (Fig. 1). Tamoxifen alone showed better effects at 48 hrs. as compared to 24 hrs. (Fig. 2). Fucoxanthin showed better effects at 0.3125 μM and Tx showed better effects at concentration of 20 μM for 24 hrs (Fig. 3). Fucoxanthin showed similar effects as Tx after 48 hrs. incubation in controlling proliferation of HeLa cells (Fig. 4).

The findings of Ye et al [10] showed an increased number of apoptotic cells to 53.4% in the early apoptosis stage after 48hrs. Our results demonstrated the number of apoptosis cells with the treatment of Fx in both early and late apoptotic events. After treating HeLa cells with 5 µM Fx for 24 hrs, the rate of early apoptotic cells was increased to 32.3% and late apoptotic cells to 23% (Fig. 7). Tamoxifen treated cancer cells showed more number of early apoptotic cells after 24 hrs (Fig. 8). Both Fx (IC50= 7.060 µM) and Tx (IC50= 5.632 µM) showed better potency against HeLa cells after 24 hrs (Fig. 5a, 6a). Few plausible mechanisms causing inhibition and apoptosis of cervical cancer cells have been proposed by previous researchers. Fucoxanthin may inhibit the growth of HeLa cells by inhibiting the P13/Akt pathway. The involvement of this pathway was suggested in cervical cancer cell proliferation, cell cycle regulation, and apoptosis. It serves a key role in the occurrence and development of a tumor.[11] NF-kB is activated in many cancers, including inflammation, proliferation, and angiogenesis. It mediates the inhibition of apoptosis by biological signals and promotes the growth of tumor cells. Fucoxanthin also reduces the activation level of NF-kB.[12]. In the present experiment, Fx induced cytoxicity in HeLa cells and the results were comparable with the effect of Tx.

CONCLUSION

The present study was performed to elucidate the in-vitro anticancer properties of Fx against HeLa cells. While anticancer properties of Fx have been proven on various cancers by previous researchers, few studies are based on cellular model. The present work proves that Fx is capable of inhibiting cell proliferation and inducing apoptosis in HeLa cells. There are certain limitations of the present study since it was focused on the role of Fx as anticancer agent against HeLa cells and not on the underlying mechanisms. Further, it is required to determine the mechanisms and pathways contributing to cell cycle regulation, inhibition of cell proliferation, and apoptosis.

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Original Article

HISTOCHEMICAL CHARACTERISTICS OF HUMAN PLACENTA IN MATERNAL HYPOTHYROIDISM

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ABSTRACT

Introduction: Maternal hypothyroidism is known to effect fetal development and maturity. These abnormalities may lead to changes in placental glycogen and lipid content that may be assessed by histochemical staining techniques. There is paucity of medical literature on histochemical changes in placenta in hypothyroidism. The present study attempts to evaluate the histochemical changes in placenta attributable to hypothyroidism as compared to euthyroid pregnancy. The main aim of the study was to study histochemical changes in placenta of normal and hypothyroid pregnancy.

Materials and methods: Fifty placentae from hypothyroid mothers (cases) and 20 from euthyroid mothers (controls) were collected after due ethical clearance from the institutional ethics committee and consent from the participants. The placental tissues were subjected to routine histological processing and stained using special stains to study the histochemical features. The slides of cases and control group were compared for degree of Periodic Acid-Schiff (PAS) and Sudan black staining to assess respectively the glycogen and lipid content in the stained tissues.

Results: Both the groups were comparable for mean age of mother, gestational age and gravida status of mother at delivery. Mild to moderate increase in the degree of staining of placental tissue with PAS and Sudan black staining was observed in hypothyroidism but the results were statistically not significant.

Conclusions: Increased glycogen and lipid content as assessed by degree of staining with PAS and Sudan black stains respectively was observed in hypothyroid placentae but it the difference between the cases and controls was insignificant. Thyroxine supplementation for variable duration during pregnancy might have led to equivocal results.

Keywords: Hypothyroidism, Normal pregnancy, Placenta, Histopathological changes

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INTRODUCTION

Placenta is a unique organ of higher mammals. It is the most significant tissue by study of which we can find out the intrauterine status of fetus and post-natal outcome. [1].

Pregnancy has a profound impact on the thyroid gland and thyroid function. Pregnancy is a stress test for the thyroid, resulting in hypothyroidism in women with limited thyroidal reserve or iodine deficiency.[2] In rats moderate maternal hypothyroidism compromises placental expression of glucose transporter (GLUT) protein isoforms and administration of T3 during late pregnancy depletes placental glycogen stores.[3] Thus maternal thyroid status may regulate placental glycogen homeostasis and glucose transport and hence the supply of maternal glucose to the fetus. Alteration in thyroid status is also associated with changes in serum triglycerides concentration and treatment by thyroid hormones preparations helps in reverting altered levels towards normal.[4]. There is paucity of medical literature on human placental changes in hypothyroid mothers. An area, which can help us to know the cause for adverse outcomes of such pregnancies. The present study attempts to evaluate histochemical changes in placenta that may be attributable to hypothyroidism.

MATERIALS AND METHODS

After due clearance from institutional ethical committee, the present prospective, observational study was carried out in the Department of Anatomy in collaboration with Department of Obstetrics and Gynaecology, at a tertiary care centre in northern India. The study was performed on 70 placentae out of which twenty belonged to mothers with uncomplicated pregnancy whereas 50 belonged to diagnosed cases of hypothyroidism (Thyroid Stimulating Hormone (TSH) level ≥3.0 IU/L) during or before pregnancy either on or without medication (thyronorm). The demographic profile and detailed history of the patient and neonate as per working proforma, were collected from hospital records, prepared and maintained in the department of gynaecology.

Placentae of mothers of diagnosed cases of fetal malformations, hypertension, anemia, diabetes and any other associated illness were excluded from the study. Placentae were procured immediately after childbirth. After cleaning and mopping, the umbilical cord was first detached from placenta. After gross examination two pieces of placental tissue each of 2 cm, one from the centre near the attachment of umbilical cord and other from periphery within 2 centimetres (cm) from the placental margin were cut and kept in 10% formalin solution for 24 to 48 hours (hrs) for histochemical study. Each beaker was labeled with a unique identification number. One section from each paraffin block of every placenta was stained with PAS stain. The slides with section were deparaffinized in xylene and rehydrated through graded alcohols to water. Then oxidized with 1% aqueous periodic acid for 5 minutes and rinsed in several changes of de-ionized water. The section was then covered with Schiff's reagent for 15 min and again rinsed in running tap water for 5-10 minutes. The sections were counter stained with Mayer's hematoxylin for differentiation and bluing and

dehydrated in graded alcohol. Cleared with xylene and mounted by using Canada balsum.[6]

For Sudan Black staining, one cryo-section which was cut with the help of cryostat machine from each placental tissue block, placed on albuminized slide, for Sudan Black staining. Two changes of Propylene glycol were made each at interval of 5 minutes. Then the section was dipped in Sudan Black stain and agitated continuously for 7 minutes. Then slide was kept in 85% Propylene glycol for 3 minutes and rinsed in distilled water. Mounting was done with aqueous mounting media, Glycerin Jelly.[7]

Periodic acid-Schiff staining was done to highlight basal membranes and extracellular matrix components of mucopolysaccharides and placental villous stroma. Glycogen deposition in basement membrane stained as magenta-colored areas while mucopolysaccaride appeared pink in color. The slides of cases and control group were compared for PAS reactivity according to intensity of staining and categorized as hazy, traces, mild and moderate.[8]

Sudan Black staining was done to see lipid deposition in the placental villi. The fat in villi appeared blue black in color. Fat cells were seen per hundred villi in each slide. According to number of fat cells per one hundred villi it was categorized as hazy, traces, mild and moderate. The photomicrographs of slides were taken by Sony digital camera with 12.1 mega pixels resolution. The preliminary processing of the data from the database was performed using the Microsoft Excel module of the Microsoft Office XP Professional software package. The statistical analysis was performed using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software. The values were represented in number (%) and Mean±SD. Student's t-test was used for comparison of cases and controls. Chi square test was used for categorical variables. A p value < 0.05 was considered significant.

Table 1. Age wise distribution of subjects

Age Group	Total	Cases (n=50)		Control (n=20)	
		No.	%	No.	%
<=20 Yrs	5	4	8.0	1	5.0
21-25 Yrs	14	7	14.0	7	35.0
26-30 Yrs	32	26	52.0	6	30.0
>30 Yrs	19	13	26.0	6	30.0
Mean Age±SD (Range)		27.82±4.05 (19-35)		27.80±5.29 (19-37)	

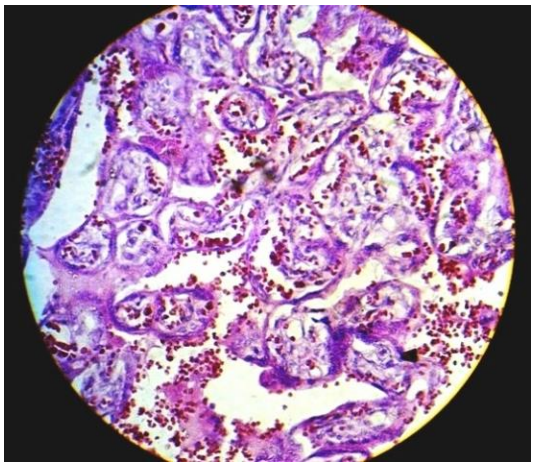

Fig 1. Photomicrograph of placenta of euthyroid mother [PAS] (40X)

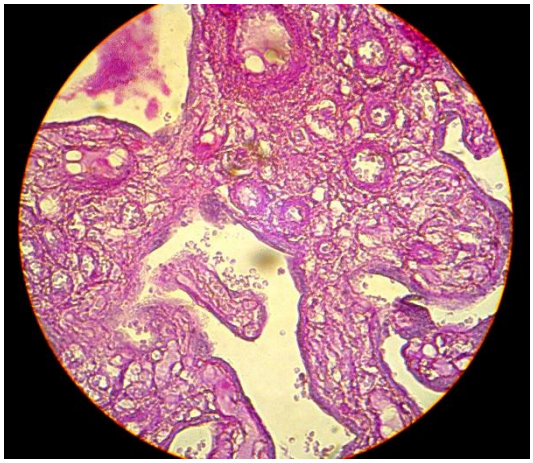

Fig. Photomicrograph of placenta of Hypothyroid mother [PAS] (40X)

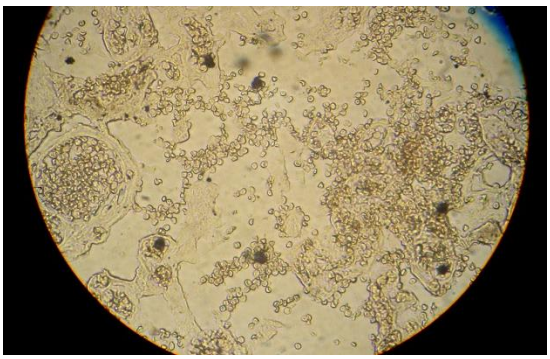

Fig 1. Photomicrograph of placenta of euthyroid mother [Sudan Black] (40X)

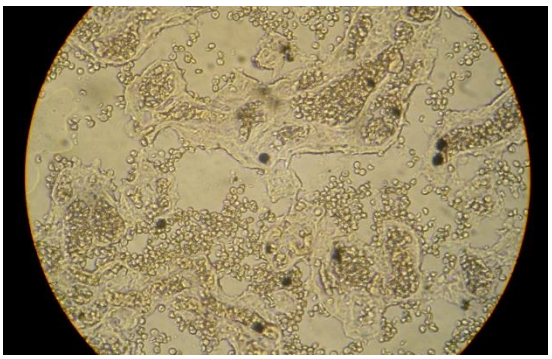

Fig. Photomicrograph of placenta of Hypothyroid mother [Sudan Black] (40X)

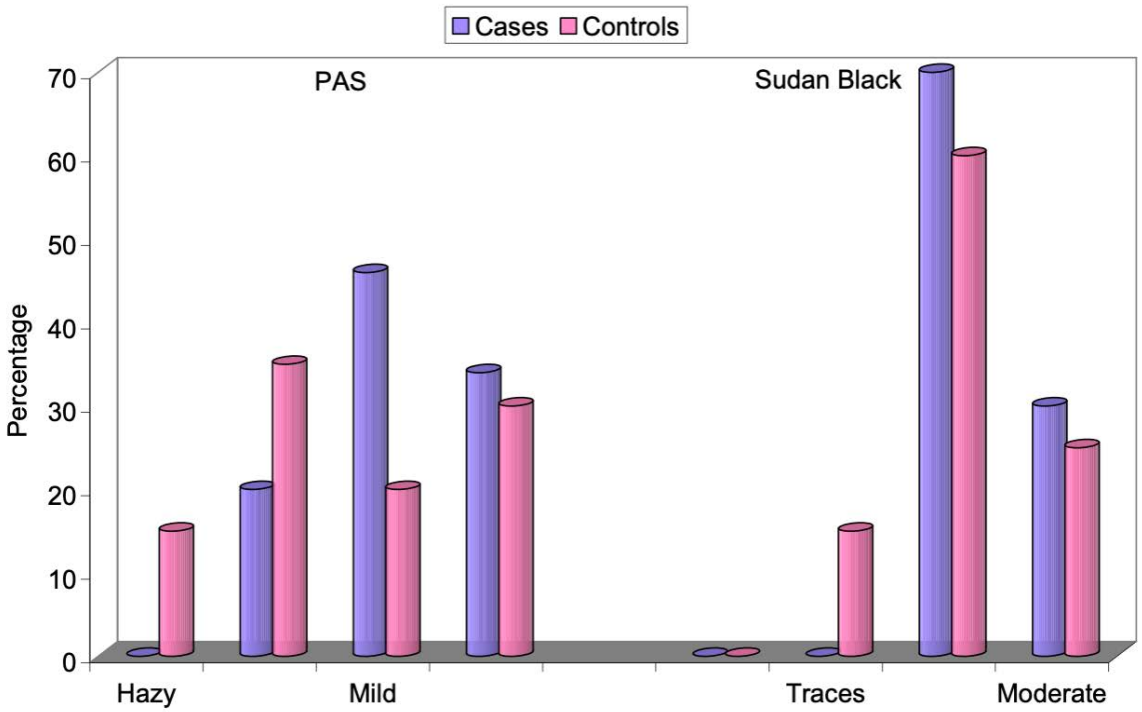

Fig 5. Bar diagram showing comparison between cases and control for the degree of histochemical staining (PAS and Sudan Black).

RESULTS

In total, 70 subjects were enrolled in the study, 50 (71.4%) placentae (case group) were obtained from women with pregnancy complicated by hypothyroidism, whereas 20 (28.6%) placentae (control group) were obtained from healthy women with uncomplicated pregnancy. Both the groups were comparable for mean age of mother (p=0.177) (Table 1), gestational age (p=0.334) (Table 2) and gravida status of mother (p=0.568) (Table 3) at delivery. The degree of PAS staining was more intense in cases as compared to controls. Mild to moderate staining with PAS was observed in 80% of the cases but only in 50% of controls (p=0.074). Similarly, the degree of Sudan black staining was more intense in cases as compared to controls. Mild to moderate staining was observed in 100% of the cases and 85% of controls (p=0.220). Despite more prominent degree of staining by both the stains in cases, the results were not statistically significant (p>0.05).

DISCUSSION

A mild to moderate enhanced glycogen storage was demonstrable in hypothyroid placentae in the present study. Maternal hypothyroidism impairs fetal growth, but the mechanism is unclear. Since the fetus derives its glucose supply from the mother, and maternal hypothyroidism may disturb maternal and placental glucose metabolism, it was postulated that placental glucose metabolic compromise may contribute to fetal growth retardation in hypothyroid fetuses.[9] In rats moderate maternal hypothyroidism compromises placental expression of glucose transporter (GLUT) protein isoforms and administration of T3 during late pregnancy depletes placental glycogen stores.[4] Thus maternal thyroid status may regulate placental glycogen homeostasis and glucose transport and hence the supply of maternal glucose to the fetus. Since glucose serves as the primary fetal growth substrate, and the fetus is incapable of gluconeogenesis[10,11], it is postulated that in

Table 2. Distribution of subjects according to gestational age of study population

GA at delivery	Total	Cases (n=50)		Control (n=20)	
		No.	%	No.	%
28-32 wks	4	3	6.0	1	5.0
32-37 wks	15	12	24.0	3	15.0
37-40 wks	44	32	64.0	12	60.0
40-41 wks	7	3	6.0	4	20.0

Table 3. Distribution of subjects according to Gravida Status of study population

Gravida	Total	Cases (n=50)		Control (n=20)	
		No.	%	No.	%
Gravida 1	32	21	42.0	11	55.0
Gravida 2	22	18	36.0	4	20.0
Gravida 3	11	8	16.0	3	15.0
Gravida 4 or above	5	3	6.0	2	10.0

Table 4. Comparison of PAS and Sudan Black Staining Results between cases and control

Test Result	Total	Cases (n=50)		Control (n=20)	
		No.	%	No.	%
PAS					
Hazy	3	0	0	3	15
Traces	17	10	20	7	35
Mild	27	23	46	4	20
Moderate	23	17	34	6	30
z=1.789; p=0.074 (Mann-Whitney U test)					
Sudan Blue					
Hazy	0	0	0	0	0
Traces	3	0	0	3	15
Mild	47	35	70	12	60
Moderate	20	15	30	5	25
z=1.227; p=0.220 (Mann-Whitney U test)					

hypothyroidism associated pregnancy, permanent fetal growth retardation may occur as a consequence of disturbances in maternal and placental glycogen storage and hence glucose supply to the fetus (i.e. maternal and/or placental glucose metabolic compromise).

The adaptations in the maternal metabolism occur throughout pregnancy that results in increased storage of fat in early pregnancy and increased availability of protein and carbohydrate in late pregnancy.[12] Alteration in thyroid status is associated with changes in serum triglycerides concentration and treatment by thyroid hormones preparations helps in reverting altered levels towards normal.[5] An insignificant rise in lipids was observed by Sudan black B staining in the placentae of thyroid hormone compromised women as compared to controls in the present study. As, all the mothers in 'case group' of present study were taking thyroid hormone substitutes, effects of altered lipid and glucose metabolism due to hypothyroidism seems to less affected as they might be partially compensated by the thyroxine replacement. Though, thyroid hormone is intricately involved in placental metabolism, but direct evidence is lacking and needs further research.

Subclinical hypothyroidism in pregnancies is a major concern, and if left untreated or undiagnosed may lead to low IQ babies. The impact of maternal hypothyroidism on placental development and therefore developing fetus needs consideration and as it is a preventable cause of infantile morbidity. The present study revealed a marginal increase in the glycogen and lipid content in hypothyroid placental tissue that may be attributable to thyroxine deficiency.

CONCLUSION

The present study compared the histochemical staining characteristics of placentae of hypothyroid and euthyroid pregnancies. The degree of staining of placental tissue with PAS and Sudan black was compared in cases and controls as a marker of glycogen and lipid content respectively. Increased degree of staining with both the stains was observed in hypothyroid placentae but the difference in the degree of staining with either of the stains in cases and controls was statistically insignificant. Thyroxine supplementation for variable duration during pregnancy may be an important confounding factor and thus a major limitation of this study.

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Original Article

SIGNIFICANCE OF ANATOMICAL VARIATIONS IN LAPAROSCOPIC CHOLECYSTECTOMY

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ABSTRACT

Introduction: Since laparoscopic cholecystectomy (LC) has emerged as the widely accepted procedure for the treatment of cholelithiasis because of its several advantages, it would be appropriate to mention the increasing incidence of bile duct injuries resulting into the most dreadful complications of the procedure. Among the various causes, anatomical variations are significant contributing factors to these injuries. So, the aim of this study was to find the incidence of abnormal anatomy and its contribution to the complications of LC.

Materials and methods: The study was conducted on 258 patients who attended the surgery OPD. The patients underwent investigations such as complete blood counts, urine examination, liver function tests, random blood sugar level and ultrasonography after a detailed history and physical examination. All the patients then underwent LC during which, the arrangement of vascular and biliary structures was noted.

Results: Of the 258 patients, 54 suffered from postoperative complications, the most common of them being biliary leakage either through the drain (33 patients) and from the abdominal cavity (16 patients). Forty-nine patients suffered from vascular and duct related complications and were subjected to MRCP. Out of these, 12 presented with accessory cystic artery while 23 showed duct anomalies such as aberrant or short cystic duct.

Conclusions: The knowledge of the normal anatomy and the various anomalies of the biliary tract and its vasculature is essential to the surgeons performing Laparoscopic cholecystectomy to reduce the incidence of postoperative complications, mortality and morbidity of patients undergoing surgery of the biliary tract.

Keywords: Laparoscopic cholecystectomy, Accessory cystic artery, Aberrant cystic duct

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INTRODUCTION

Laparoscopic cholecystectomy (LC) has replaced the open surgical approach for the treatment of cholelithiasis worldwide because of its several advantages. LC is associated with reduced pain and the need for analgesia, which in turn decreases the duration of hospital stay and the patient becomes fully active within one week as compared to one month after open cholecystectomy. It also provides improved cosmesis and patient satisfaction as compared to open cholecystectomy.[1]

However, adopting LC as a new technique for treatment of cholelithiasis, has introduced a new spectrum of complications. A higher incidence of bile leaks and injuries to the common bile duct was observed as compared to the procedure of open cholecystectomy.[2-4]

Laparoscopic cholecystectomy is associated with an overall complication rate of approximately ten percent with a higher risk of biliary injury (0.1%-1.5%) when compared to the open approach (0.1%-0.2%).[5]

These complications are life threatening and highly affect the quality of life.

There are several risk factors which can contribute to iatrogenic injury of the biliary tract:

- anatomical factors
- patient-related factors
- factors related to gallbladder disease
- the surgical technique
- and the surgeon.[6]

Several anatomic variations of the biliary tract and hepatic vessel and its branches increase the risk of iatrogenic lesions during LC mainly in the presence of acute inflammation.[7]

These anatomical variants of the biliary tract include, for example, the different variants of the cystic duct, such as a short cystic duct, cystic duct running parallel to the CBD, anomalies of the CD-CHD junction, presence of the hepatocystic duct, accessory cystic duct, the existence of aberrant bile ducts (ex. Luschka duct), etc.

Among the patient-related factors, severe obesity, previous surgery on the biliary tract and underlying liver diseases seem to be the predisposing factors for peri-operative complications.[6]

Although, LC has been widely accepted as the standard of care, it continues to have a higher complication rate than open cholecystectomy. Bile duct injury with LC has often been attributed to surgical inexperience, but it is also clear that aberrant bile ducts are present in a significant number of patients who sustain biliary injuries during these procedures.[8]

So, the aim of this study was to correlate the anatomical variant factors with the complications of Laproscopic Cholecystectomy.

MATERIALS AND METHODS

The study was conducted in the surgery department of NCR Institute of Medical Sciences, Meerut between the years 2017-2020. In this study, 258 patients were included who attended the surgery OPD, their detailed history was taken followed by clinical examination of all patients.

The required investigations including complete blood count, urine examination, liver function tests, random blood sugar levels, and ultrasonography were done. Patients who were obese, having any previous surgery on the biliary tract or any underlying liver disease were excluded from the study to minimize the post-operative complications. After obtaining the informed consent, all the patients underwent Laparoscopic Cholecystectomy. During the surgery, the arrangement of vascular and biliary structures was noted. Patients were then followed up for the various post-operative complications.

RESULTS

Out of 258 patients operated for LC, there were 54 patients with postoperative complications. The most common postoperative complications were: bile leaks through the drain > 100 ml/24h (in 33 patients), bleeding from the abdominal cavity (in 16 patients). A less frequent complication was surgical wound infection (in 5 patients).

The optimal treatment protocol was followed either by T- Tube repair or hepaticojejunostomy depending upon the ductal injury.

Out of 49 patients who presented with vascular and duct related complications, an investigational approach using MRCP was followed. In the study 12 patients were found to have accessory cystic artery and 23 patients presenting with bile leaks were found to have ductal anomalies as shown in Table 1.

DISCUSSION

LC is one of the most commonly performed surgical procedures nowadays, therefore, the complications associated with this procedure have also risen. Ductal injuries are the most common ones followed by vascular anomalies. Although there were various risk factors associated with the complications of LC, variance in the normal anatomy of the biliary tract affected the morbidity and mortality of the patients.

The experience of the surgical team with the operative technique and equipment and the relationship dynamics with other team members were important factors in preventing the complications.[9-13]

P V Suhocki et al (1999) found seventeen percent (14/82) of the patients with aberrant bile duct anatomy. Fifteen percent (12/82) were found to have an aberrant bile duct involved in the injury thus increasing the complications.[14]

R B Adkins et al(2000) specified that the success and safety of laparoscopic cholecystectomy, orthotopic liver transplantation, and trisegmentectomy for hepatic tumors depend on accurate knowledge of the anatomy and embryologic anomalies of the biliary tree. They are the source of major challenge to unprepared and unaware surgeons.[15]

M Lamah et al (2001) focused on the sound knowledge of the normal anatomy of the extrahepatic biliary tract in the prevention of operative injury to it and also emphasized on the understanding of congenital variation of biliary and vascular anatomy, as the literature had abundant reports of specific anatomical variations, and their operative implications.[16]

Sanjay Nagral et al (2005) concluded that misinterpretation of normal anatomy and anatomical variations contribute to the

Table 1. Number of anomalies found

S. No.	Anomalies found	No. of patients	Percentage	
1	Accessory cystic artery	12	4.65%	
2	Aberrant cystic duct	13	5.03%	8.9%
3	Short cystic duct	10	3.87%	

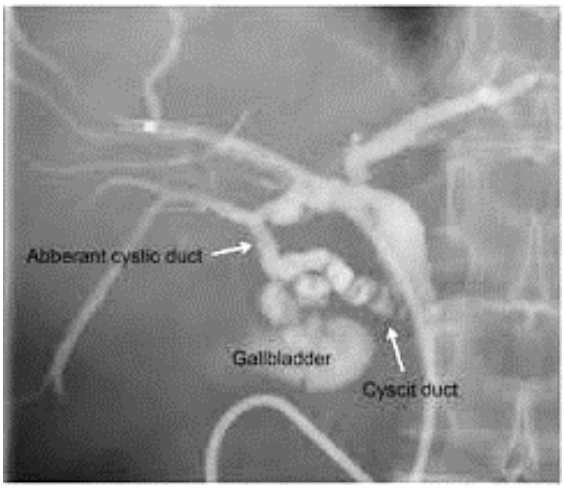

Fig. 1. MRCP showing aberrant cystic duct

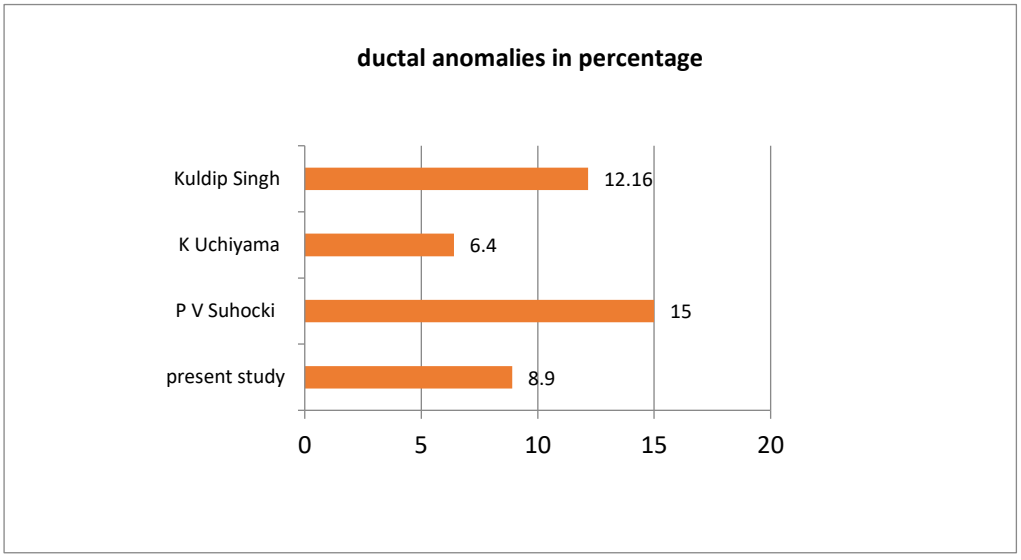

Fig 2. Comparison of the ductal anomalies of present study with the previous literature

occurrence of major postoperative complications like biliary injuries following a cholecystectomy, the incidence being higher with laparoscopic cholecystectomy.[17]

K Uchiyama et al (2006) clearly found secondary branches of aberrant cystic ducts in 37 cases (3.5%), and accessory hepatic ducts in 30 cases (2.9%) and emphasized on the preoperative evaluation of the bile duct tract and the accessory hepatic duct before LC to avoid difficulty in during the surgery and its complications.[18]

Ahmad Hassan Khan etal (2008) found that out of 100, 80 patients were females while the rest were males. Majority of the patients were in their fourth and fifth decades of life. Moynihan's hump and accessory cystic artery were the most frequently found anomalies (n=6 each), followed by a double cystic duct and a long cystic duct and concluded that extra hepatic biliary tree anomalies were found in a significant number of patients. A sound knowledge of their normal anatomy, various anomalies and judicious use of intra-operative cholangiography were imperative to avoid accidental trauma to the extrahepatic biliary channels and vessels.[19]

Kuldip Singh et al (2017) revealed that among the seven hundred forty cases of cholelithiasis, irrespective of pathology, 197 (26.62%) vascular anomalies and 90 (12.16%) ductal anomalies20 were observed.

CONCLUSION

On the basis of the present study and the previous literature, it can be concluded that the normal anatomy and the various anomalous features of the biliary tract and its vasculature should be meticulously known to the surgeons to prevent postoperative complications of Laparoscopic cholecystectomy arising due to the lack of knowledge regarding the anomalies thus minimizing the complications and reducing morbidity and mortality of patients.

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Original Article

LEVEL OF AWARENESS ABOUT MEDICAL ETHICS AMONG JUNIOR DOCTORS OF A TERTIARY CARE HOSPITAL IN NORTH INDIA - A CROSS-SECTIONAL STUDY

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ABSTRACT

Introduction: The practice of medicine is bound by ethics. There are well established ethical frameworks, i.e. "Code of medical ethics of India" and a regulatory body like "Medical Council of India". The doctors need to follow certain norms while dealing with patients and his/her relatives. But failure to do so advertently or inadvertently may lead to patient dissatisfaction or litigation in the court of law. Our study aimed to check the level of understanding about medical ethics among 100 junior doctors of a tertiary care hospital in north India.

Materials and methods: The sample size was kept as 100 junior doctors viz. 43 interns and 57 postgraduate (PG) students (both clinical and non-clinical). They were asked to fill the consent forms before the study and were given questionnaires to attempt. Their answers were statistically analysed.

Results: It was observed that 29.8% PGs and 2.3% interns were unaware of the basis for "International Code of Medical Ethics" (statistically significant); 80.7% PGs and 74.4% interns were aware of the practice of writing generic names of drugs in prescription; 45.6% PG students, 32.6% interns had good knowledge about the points to be covered while taking "Informed Consent"; 59.6% PG students, 37.2% interns were aware of "medical negligence" with p-value=0.043 (statistically significant).

Conclusions: PG students have more knowledge about the components of medical ethics which are to be followed during their everyday clinical practice than interns who have more theoretical knowledge. Thus, there is a need to refresh their understanding of medical ethics.

Keywords: Medical ethics, Resident doctors, Post Graduate students, Interns

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INTRODUCTION

Everything a doctor does in medicine is bound by ethics and he has taken oath regarding it. During patient care and management, doctors need to follow certain norms. However, failure to do so can lead to patient dissatisfaction or legal action. Medical ethics is the study of morality in medicine, which is concerned with the doctor and patient relationship. Whatever his/her specialty, every doctor has to discharge medico-legal responsibilities and solve medico-legal problems from the very first day of his/her practice and do no harm. Hence, with this increased awareness of an individual's rights, doctors need to revisit the subject of medical ethics.

In recent times, under the influence of newspapers, T.V. and internet, the patients demand to be fully informed about options, possible complications and other aspects of the treatment plans offered, due to increase in their awareness on the same. The fact that after every day or two, numerous reports are seen in the newspapers regarding the misconduct of ethics by doctors also contributes to this. In light of this lack of knowledge of medical ethics and its significance in enhancing physician-patient ties, doctors need to revisit the subject of medical ethics. Numerous studies have suggested that doctors in routine clinical practices do not obtain proper consent after providing thorough information or just getting the consent signed without giving any information.

There are well established ethical frameworks in the field of medicine, i.e. Code of Medical Ethics of India, State Medical Council and regulatory bodies like Medical Council of India (MCI), now replaced by National Medical Commission (NMC). Despite this fact, no significant steps have been taken to ensure its proper implementation and its continuance as a part of medical education at undergraduate (UG) or postgraduate (PG) level. It may improve awareness about the practice of medical ethics among junior doctors. The present study will reveal the hidden problem of improper implementation of medical ethics by the doctors in their medical practice.

MATERIALS AND METHODS

The present study was a cross-sectional, observational study conducted at Subharti Medical College and Chhatrapati Shivaji Subharti Hospital, Meerut, in collaboration with the Department of Community Medicine, Subharti Medical College, Meerut. Our study aimed to check the level of awareness about medical ethics among 100 junior doctors of the hospital. The study required minimal resources like consent forms and pre-designed questionnaires. The sampling technique employed was purposive. We included all interns and PG students (both clinical and non-clinical) except who were unwilling to fill the questionnaire or were not available at the time of data collection.

We structured the questionnaires in such a manner to collect data for independent variables like sex (male/female) and designation (PG student/intern) and dependent variables like Declaration of Geneva, privileged communication, prescription of drugs, informed

consent, MTP, covering, medical negligence, advertisement, dichotomy (fee-splitting), use of Red Cross Emblem and confidentiality. This enabled us to gather data comprising of almost all essential aspects of medical ethics followed in daily practice.

METHOD:

Informed consent from all the subjects and clearance from the medical college's institutional ethics committee was obtained. Questionnaires were given to the junior doctors. They were asked about their knowledge regarding the essential elements of medical ethics like informed consent, confidentiality, dichotomy (fee-splitting), adultery, advertisement, etc. The answers were analyzed to assess the level of awareness about medical ethics among them. Statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 24.0 (Chicago, Inc, USA). Chi-Square test was used to identify the significance of the association between the level of awareness and selected factors. Then, conclusions and interpretations were drawn from the analysed data.

RESULTS

The present study aimed to check the level of awareness about the various aspects of medical ethics among 100 junior doctors of the tertiary care hospital. The participants included interns and PG students (both clinical and non-clinical) available in the hospital at the time of collection of data. The study population's demographic profile revealed that the majority (50%) of the participants were in the age group of 24-26 years (Table 1, Fig. 1).

Age of participants of the study ranged between 21 and 32 years. A majority (50%) of the participants were in 24-26 years.

Subsequently, answers from the questionnaires were statistically analyzed. They revealed important relationships between level of awareness regarding the essential elements of medical ethics like informed consent, confidentiality, privacy, adultery, advertisement, etc. and designation or sex (male/female). Following tables (Tables 2-6) show the statistical analysis for the same.

Table 1. Demographic profile of study population (n=100)

SN	Characteristics	Statistics (Frequency)
1.	**Gender**	
	Female	43 (43.0%)
	Male	57 (57.0%)
2.	**Age (years)**	
	21-23	27 (27.0%)
	24-26	50 (50.0%)
	27-29	18 (18.0%)
	30-32	5 (5.0%)
	Total	100 (100.0%)
3.	**Designation**	
	Postgraduate students	57 (57.0%)
	Interns	43 (43.0%)
	Total	100 (100.0%)

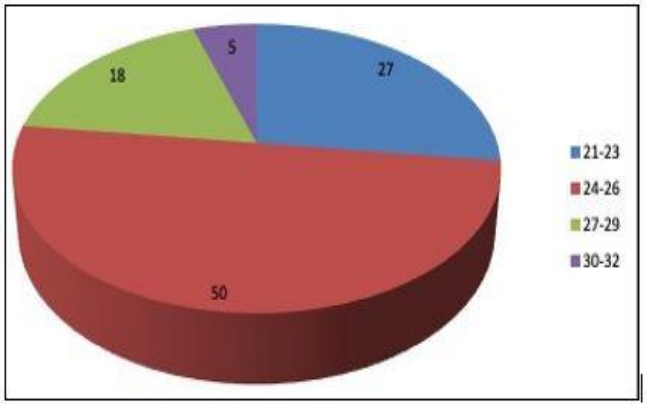

Fig. 1. Distribution of study subjects as per different age

Table 2. Relationship between level of awareness about the basis for the international code of medical ethics, privileged communication and designation

Designation	International Code of Medical Ethics					Total
	Dec. Oslo	Dec. Tokyo	Dec. Helsinki	Dec. Geneva	Don't know	
Postgraduate students	4	1	8	40	4	57
	7.0%	1.8%	14.0%	70.2%	7.0%	100.0%
Interns	0	0	1	42	0	43
	0.0%	0.0%	2.3%	97.7%	0.0%	100.0%
Total	4	1	9	82	4	100
	4.0%	1.0%	9.0%	82.0%	4.0%	100.0%
Test of significance	X^2=12.784, df=4, p=0.012					
Designation	**Privileged Communication**					**Total**
	Concerned authority for interest of community	Patient for the interest of community	Concerned authority for interest of patient	Don't know		
Postgraduate students	24	11	15	7		57
	42.1%	19.3%	26.3%	12.3%		100.0%
Interns	20	12	8	3		43
	46.5%	27.9%	18.6%	7.0%		100.0%
Total	44	23	23	10		100
	44.0%	23.0%	23.0%	10.0%		100.0%
Test of significance	X^2=2.221, df=3, p=0.528					

Among PG students, 70.2% were well aware of the nternational code of medical ethics, while 97.7% interns knew about it. This association was statistically significant (p=0.012). 42.1% PGs and 46.5% interns knew about privileged communication and this association was statistically insignificant (p-value>0.05) (Table 2).

80.7% PG students and 74.4% interns knew the correct answer about practice of prescription writing. This association was not statistically significant (p=0.642). Among PG students, 45.6% while among interns, 32.6% were aware of informed consent. This association was statistically significant (p=0.006) (Table 3).

Among female doctors, 44.2% knew well about the ethical practice of issuing a death certificate, whereas among male doctors, 33.3% were aware about it. The association was statistically insignificant (p-value>0.05). With regards to the issuance of a medical certificate, 79.1% female doctors and 75.4% male doctors, knew the right answer. This association was statistically insignificant (Table 4).

Table 3. Relationship between practice of writing prescription (names of drugs), taking informed consent and designation

Designation	Prescription (Names of drugs)				Total
	Trade name	Brand name	Generic name	Don't know	
Postgraduate students	5	5	46	1	57
	8.8%	8.8%	80.7%	1.8%	100.0%
Interns	6	5	32	0	43
	14.0%	11.6%	74.4%	0.0%	100.0%
Total	11	10	78	1	100
	11.0%	10.0%	78.0%	1.0%	100.0%
Test of significance	X^2=1.677, df=3, p=0.642				

Designation	Informed consent			Total
	Didn't answer	Answered correct	Answered wrong	
Postgraduate students	1	26	30	57
	1.8%	45.6%	52.6%	100.0%
Interns	9	14	20	43
	20.9%	32.6%	46.5%	100.0%
Total	10	40	50	100
	10.0%	40.0%	50.0%	100.0%
Test of significance	X^2=10.241, df=2, p=0.006			

Table 4. Relationship between the ethical practice of issuing a medical certificate, a death certificate and sex

Sex	Death Certificate in Poisoning					Total
	Yes, with reason	Yes, without reason	No with reason	No without reason	Don't know	
Female	1	5	19	15	3	43
	2.3%	11.6%	44.2%	34.9%	7.0%	100.0%
Male	5	5	19	28	0	57
	8.8%	8.8%	33.3%	49.1%	0.0%	100.0%
Total	6	10	38	43	3	100
	6.0%	10.0%	38.0%	43.0%	3.0%	100.0%
Test of significance	X^2=7.790, df=4, p=0.100					

Sex	Medical Certificate to Child				Total
	Yes, with reason	Yes, without reason	No	Don't know	
Female	3	6	34	0	43
	7.0%	14.0%	79.1%	0.0%	100.0%
Male	5	7	43	2	57
	8.8%	12.3%	75.4%	3.5%	100.0%
Total	8	13	77	2	100
	8.0%	13.0%	77.0%	2.0%	100.0%
Test of significance	X^2=1.702, df=3, p=0.636				

Table 5. Relationship between awareness about the indications of MTP, Declaration of Oslo, medical negligence and designation

Designation	**Therapeutic abortion**			**Total**
	Yes, with indications	Yes, without indications	No	
Postgraduate students	33	14	10	57
	57.9%	24.6%	17.5%	100.0%
Interns	18	5	20	43
	41.9%	11.6%	46.5%	100.0%
Total	51	19	30	100
	51.0%	19.0%	30.0%	100.0%
Test of significance	X^2=10.249, df=2, p=0.006			

Designation	**Medical negligence**					**Total**
	Duty owed to the patient	Dereliction of duty	Damage to patient	Error of judgment	Don't know	
Postgraduate students	10	5	2	34	6	57
	17.5%	8.8%	3.5%	59.6%	10.5%	100.0%
Interns	19	5	1	16	2	43
	44.2%	11.6%	2.3%	37.2%	4.7%	100.0%
Total	29	10	3	50	8	100
	29.0%	10.0%	3.0%	50.0%	8.0%	100.0%
Test of significance	X^2=9.839, df=4, p=0.043					

Table 6. Relationship between level of awareness about taking written consent from victim/patient, confidentiality and designation

Designation	Written consent mandatory					Total
	Examination of rape victim	Abortion	Family planning operation	Injecting medication	Don't know	
Postgraduate students	2	2	3	47	3	57
	3.5%	3.5%	5.3%	82.5%	5.3%	100.0 %
Interns	2	2	5	32	2	43
	4.7%	4.7%	11.6%	74.4%	4.7%	100.0 %
Total	4	4	8	79	5	100
	4.0%	4.0%	8.0%	79.0%	5.0%	100.0 %
Test of significance	X^2=1.620, df=4, p=0.805					

Designation	Medical Details of Patient to Investigation Officer				Total
	Yes, with reason	Yes, without reason	No with reason	No without reason	
Postgraduate students	10	8	19	20	57
	17.5%	14.0%	33.3%	35.1%	100.0%
Interns	4	18	7	14	43
	9.3%	41.9%	16.3%	32.6%	100.0%
Total	14	26	26	34	100
	14.0%	26.0%	26.0%	34.0%	100.0%
Test of significance	X^2=11.276, df=3, p=0.010				

Among PG students, 57.9% were aware of the indications of MTP & Declaration of Oslo, whereas among interns, 41.9% were conscious regarding this. This association was statistically significant (p=0.006). 59.6% PGs knew about medical negligence, while among interns, 37.2% were aware of it. The association was statistically significant (p=0.043) (Table 5).

82.5% PGs knew the correct answer which formed the basis of written consent, while among interns, 74.4% knew about it. The association was statistically insignificant (p-value>0.05). 33.3% PG students and 16.3% interns knew the correct answer, which showed their awareness about the ethics regarding confidentiality. This association was statistically significant (p=0.010) (Table 6).

Awareness regarding some other aspects of medical ethics like the opening of own shop of medicines and surgical instruments next to one's own nursing home, 23.3% female doctors and 31.6% male doctors were in favour. The association was statistically insignificant. 59.6% PG students and 67.4% interns were aware of drugs which were banned by the government. The association was not statistically significant. 76.7% of female doctors knew the correct answer, which formed the basis of covering, whereas 64.9% of male doctors knew about it. The association was statistically insignificant (p-value>0.05). Among PG students, 68.4% knew about the ethics regarding advertisement, whereas among interns, 60.5% were aware. The association was statistically insignificant. Awareness about dichotomy was more evident among 45.6% PGs and 41.9% interns and this was statistically insignificant (p-value>0.05).

Among PG students, 40.4% knew about the use of Red Cross Emblem, whereas among interns, 18.6% were aware. The association was statistically significant (p=0.004).

DISCUSSION

During this survey involving 43 interns and 57 PG students (i.e., a sample size of 100) both from clinical and non-clinical departments of Subharti Medical College and Hospital, it was found that 27% of study subject fell in the age group 21-23 years, 50% fell in the age group 24-26 years and rest 23% were above 27 years. Among the study subjects, 43% were female doctors and 57% were male doctors. In the current study, based on the answers received from the questionnaires, the level of awareness was inferred. Unnikrishnan et al. (2014) also used a semi-structured questionnaire to collect the data via a 5-point Likert scale and made inferences based on those responses [1]. Among 100 study subjects, 49% attended oath ceremony and the rest 51% didn't in our study. In comparison to Jalal et al. (2018), survey results showed that 9% of doctors were not familiar with the key contents of the Hippocratic Oath and 89% of these were junior doctors [2].

In our study, after a thorough statistical analysis, it was found that 29.8% PG students and 2.3% interns were not aware of the basis for "International Code of Medical Ethics". This assertion was statistically significant (p=0.012), showing that fewer PG students were mindful of the "International Code of Medical Ethics" from our studied group. Also, 57.9% of PG students and 53.5% of interns were not aware of

"Privileged Communication". We found that 80.7% PG students and 74.4% interns were mindful of the apt practice of prescribing names of drugs, i.e. generic names should be prescribed. In contrast, Chatterjee & Sarkar (2012), found that only 50.6% of doctors were aware of prescribing generic names of drugs [3]. Thus, there is a higher level of awareness amongst our study subjects.

It was also observed that 40% of the total 100 doctors, of which 45.6% PG students, 32.6% interns had good knowledge about the points to be covered while taking "Informed Consent". This was statistically significant with p=0.006; on comparison, in a study by Qidwai et al. (2013), 66.4% of the total patients reported that doctors had taken informed consent [4]. Rai et al. (2012) reported that nearly 90% of the respondents were aware of informed consent [5]. Thus, we need to mandate upon inculcating this as a routine protocol to increase the awareness of the same. Furthermore, 74.4% females and 63.2% males strongly disagreed about opening own shop of medicines and surgical instruments. Hence, 68% from the total studied group disagreed about opening own shop of medicines and surgical instruments, while Chatterjee & Sarkar (2012) found only 46.3% disagreed with opening the shop [3]. Thus, our study showed more ethical awareness amongst doctors who strongly disagreed about opening the shop.

62% of the total doctors (55.8% females and 66.7% males) had no idea about the ethical practice of issuing a death certificate. In a study by Shreemanta (2010), 68% of the participants stated that they were not aware of the international format of Death Certification [6]. Thus, our study revealed comparable data with the other study. 42.1% PG students and 58.1% interns were not aware of the "Indications of MTP & Declaration of Oslo". This difference was statistically significant (p=0.006). Concerning gender, there was a statistically insignificant difference (79.1% females and 75.4% males) as both knew well regarding the ethical practice of issuing a medical certificate. 59.6% PG students and 67.4% interns were well aware of the banned drugs. 76.7% females and 64.9% males knew well about covering.

59.6% PG students were aware of medical negligence, while among interns, only 37.2% knew well about it. This difference was statistically significant (p=0.043). Varghese et al. (2016) stated that most of the interns (61.3%) lacked proper knowledge about medical negligence and medical ethics, whereas 48.1% of resident doctors had adequate knowledge [7]. Thus, this topic needs better address via medical education CME's or programs to strengthen its understanding. 82.5% PG students and 74.4% interns were well aware of taking written consent from a victim or patient. 45.6% PG students and 41.9% interns knew about "Dichotomy". We found that 68.4% PG students and 60.5% interns knew that "Advertisement" is not ethical. Chatterjee & Sarkar (2012) reported that only 52.2% of interns were aware of it [3]. Thus, our study showed a higher awareness level about the ethics regarding advertisement.

33.3% PG students and 16.3% interns knew how and when to keep the information confidential, while as per Chatterjee & Sarkar

(2012), 77.3% interns knew about "Confidentiality" [3]. Moreover, Rajput et al. (2017) revealed 71.4% students, 51.8% interns and 26.7% residents agreed upon confidentiality [8]. Fadare et al. (2012) deduced that confidentiality was recognized by only 53.4% of doctors who participated in the study [9]. Thus, our PG students and interns need more workup regarding "Confidentiality" as keeping information confidential is an important component for maintaining doctor-patient relationships. Among PG students, 40.4% were conscious regarding the use of Red Cross Emblem, whereas only 18.6% interns could answer the question regarding it correctly. Similar to our findings, Shreemanta (2010) inferred that 43% practitioners knew well about its use [6].

Ranasinghe et al. (2020) concluded that majority of the doctors (81.2%) lacked an adequate level of understanding on medical ethics, with PGs showing significantly (p=0.023) higher level of knowledge [10]. This finding is in line with the results of our study.

Nonetheless, there is a need to encourage the importance of basic principles of medical ethics, i.e. informed consent, privacy, confidentiality, advertisements, dichotomy, medical negligence & Declaration of Geneva. In their services, they should receive regular CMEs (Continued Medical Education) to emphasize fundamental principles of medical ethics like privacy, confidentiality, need of taking informed consent, unethical advertisements, medical negligence, prescribing generic names of drugs, etc. This would enable health providers to resolve any ethical issues that frequently arise in clinical settings.

CONCLUSIONS

This shows that PG students have more knowledge about the components of medical ethics which are to be followed during their interaction with patients (everyday clinical practice) viz. the practice of writing a prescription, points to be covered while taking informed consent, declaration of Oslo & Indications of MTP, medical negligence, advertisement being unethical, use of Red Cross Emblem, dichotomy and confidentiality; as compared to interns who have more of theoretical knowledge viz. privileged communication, International Code of Medical Ethics and banned drugs. Thus, there is a need to refresh their understanding of medical ethics. We can also state that female doctors had more knowledge than male doctors about the components of medical ethics viz. opening own shop of medicine and surgical instruments, issuing of a death certificate, ethical practice of issuing medical certificate & covering. This shows that female doctors have much more ethical awareness and judgment as compared to male doctors.

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Original Article

TOPOGRAPHICAL AND RADIOLOGICAL EVALUATION OF LATERAL PTERYGOID MUSCLE

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ABSTRACT

Introduction: Masticatory muscles play an important role in various activities like mastication, pronunciation, swallowing and speech. Lateral pterygoid is one of the four principal muscles of mastication. Earlier studies have shown that the lateral pterygoid muscle takes origin from the cranium and inserts on the mandible and articular disc of the temporo-mandibular joint.

Materials and methods: Careful dissection was carried out on thirty embalmed adult cadaveric head-halves while preserving the inferior alveolar vessels and nerve, lingual nerve, buccal nerve and maxillary artery in order to examine the anatomical details of the lateral pterygoid muscles. Anatomical features of the lateral pterygoid muscles were recorded. Lateral pterygoid muscles were identified in the MRI scans of adult patients and their anatomical features were also recorded.

Results: The observations recorded in the present study entail topographic assessment of the lateral pterygoid muscle on cadaveric dissection and MRI scans of the adult patients. Lateral pterygoid muscle consists of two heads – upper and the lower head. The upper head arises from the infratemporal surface of the greater wing of the sphenoid bone, while the lower head arises from the lateral surface of the lateral pterygoid plate. It is inserted into the front of the neck of the mandible and articular disc of the temporo-mandibular joint.

Conclusions: This study aims to provide detailed information regarding anatomical profile of lateral pterygoid muscles, thereby helping dental and maxillo-facial surgeons in their operative procedures. Thus, basic anatomical knowledge of the attachment of the lateral pterygoid muscle would enhance the diagnostic and surgical skills of both dental and maxillo-facial surgeons.

Keywords: Lateral pterygoid, Pterygoid plates, Mandible

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INTRODUCTION

The four principal muscles of mastication are – masseter, temporalis, lateral pterygoid and medial pterygoid.[1] Lateral pterygoid muscle consists of two heads – upper and the lower head. The upper head arises from the infratemporal surface of the greater wing of the sphenoid bone, while the lower head arises from the lateral surface of the lateral pterygoid plate. The two heads converge as they pass backwards to insert into the front of the neck of the mandible and articular disc of the temporo-mandibular joint. When the bilateral muscles contract together, they protrude the mandible and depress the chin and thus assist in the opening of the jaw. Additionally, the lateral pterygoid participates in chewing movements along with the medial pterygoid muscle.[2]

Clinical evaluation of the infratemporal fossa is cumbersome as the contents are situated at the craniofacial junction. Therefore, MRI is considered as one of the best techniques of imaging due to its accuracy in the evaluation of the soft tissues. Additionally, these anatomical and radiological studies help the maxillofacial surgeons to perform safe surgery of the infratemporal fossa avoiding damage to the vital structures.[3] Therefore, basic anatomical knowledge would enhance the diagnostic skills of both the oral and maxillo-facial surgeons.

Spasm of the lateral pterygoid muscle can give rise to tenderness on palpation behind the maxillary tuberosity in temporomandibular joint disc syndrome. This is known as the pterygoid sign.[1] Therefore, the present study was performed to examine the attachments and orientation of the fibres of the lateral pterygoid muscles through the cadaveric dissection and MRI scans of subjects of Indian origin.

MATERIALS AND METHODS

The study was carried out on thirty embalmed adult cadaveric head-halves in order to examine the anatomical details of the lateral pterygoid muscles.

The cadavers were placed in supine position. Skin incision was given in the midline from the forehead to the mental protuberance passing through the nasion encircling the mouth. Another incision was given from the mental protuberance along the inferior border of the mandible to the ear lobes. A circular incision was made around the orbit to the upper border of the external ear. All the skin flaps were reflected and superficial fascia along with the parotid gland and the masseter muscle was removed. The zygomatic arch was cut using the probe and saw and was reflected inferiorly along with the masseter muscle. The ramus of the mandible was then removed in order to expose the lateral pterygoid muscles. A horizontal cut was made through the neck of the mandible and another cut was given just above the mandibular foramen. The lateral pterygoid muscles were exposed deep to the mandible and anatomical details of the lateral pterygoid muscles were studied.

Thirty adult patients were scanned by MRI using Standard head protocol which included the axial images in T1, T2 and FLAIR sequences. Slice thickness of 4 mm with 1 mm gap was used. Images were obtained using 1.5 Tesla Philips Achieva Scanner with sense head coil. Maximum thickness of the lateral pterygoid muscles was evaluated at the level of clivus.

Anatomical features of the lateral pterygoid muscles were recorded. Data was entered in MS Excel and statistical analysis was performed using SPSS version 21. Quantitative data was presented as table and bar diagram.

RESULTS

Observations about the lateral pterygoid muscle on cadaveric dissection

(a) Anatomical features – The lateral pterygoid was observed as a short thick muscle. Two heads of the lateral pterygoid muscle – upper and the lower head were found in all of the specimens examined (Fig. 1).

(i) Cranial attachment – In the present study, both the heads of the lateral pterygoid muscles were found to have a different site of cranial attachment in all the specimens studied. The cranial attachment of the upper head of the lateral pterygoid muscle was found to be at the infratemporal surface and the infratemporal crest of the greater wing of the sphenoid bone. The cranial attachment of the lower head of the lateral pterygoid muscle was found to be at the lateral surface of the lateral pterygoid plate of the sphenoid bone.

(ii) Mandibular attachment – The mandibular attachment of the lateral pterygoid muscle was observed at the pterygoid fovea in front of the neck of the mandible. However, few of the upper fibres of the lateral pterygoid muscle were found to be attached at the articular disc and capsule of the temporomandibular joint in 24 of 30 specimens studied. Furthermore, in 6 of the specimens, it was noticed that the all the fibres of the lateral pterygoid muscle were attached solely on to the neck of the mandible.

(iii) Orientation of fibres – Both upper and the lower heads of the lateral pterygoid muscle were found to converge and were found to pass backwards and laterally. The fibres of the lateral pterygoid muscle were found to be musculo-aponeurotic in profile in all of the specimens examined.

(iv) Relations with nerves and vessels – Inferior alveolar nerves were seen emerging from the lower border of the lateral pterygoid muscle which traversed downwards and forwards and entered the mandibular foramen. They were seen to be accompanied by the inferior alveolar vessels (Fig. 1). The buccal nerve emerged between the two heads of the lateral pterygoid muscle which was observed to pass downwards and forwards.

Masseteric nerve was seen to pass superior and lateral to the lateral pterygoid muscle behind the tendon of the temporalis. Then it was seen to traverse through the mandibular notch along with the masseteric vessels.

The maxillary artery was observed to traverse along the lower border of the upper head of the lateral pterygoid muscle. It passed upwards and forwards superficial to the lateral pterygoid muscle and then it coursed between the two heads of the lateral pterygoid muscle to reach the pterygopalatine fossa (Fig. 1).

(v) Innervation – The lateral pterygoid muscle was found to be innervated by the branches of the anterior division of the mandibular nerve in all of the specimens studied.

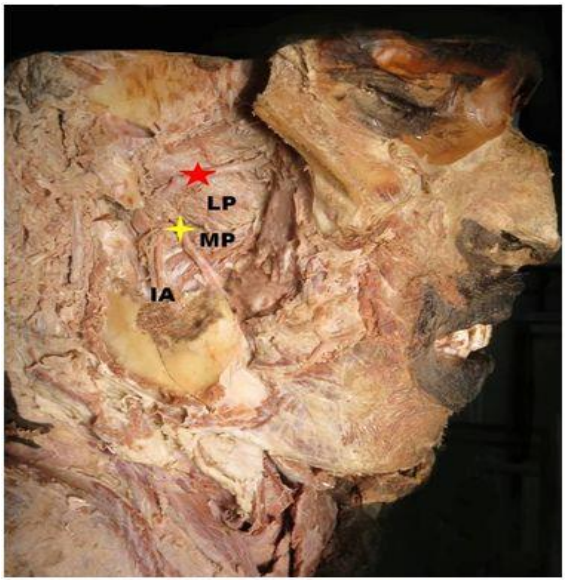

Fig 1. Lateral pterygoid muscle and its relations
LP – Lateral pterygoid muscle;
MP – Medial pterygoid muscle;
IA – Inferior alveolar nerve and vessels;
★ Maxillary artery; ✦ Lingual nerve

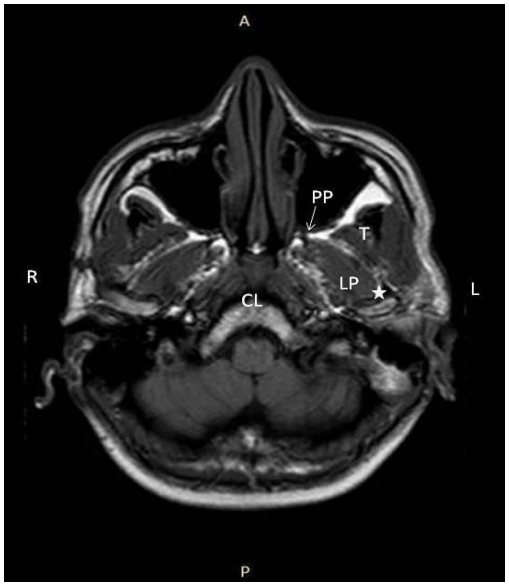

Fig 2. MRI scan of head region (T1 – axial image);
LP – Lateral pterygoid muscle;
T – Temporalis muscle; CL – Clivus;
PP – Pterygoid plate of Sphenoid bone;
☆ - Pterygoid fovea of the mandible

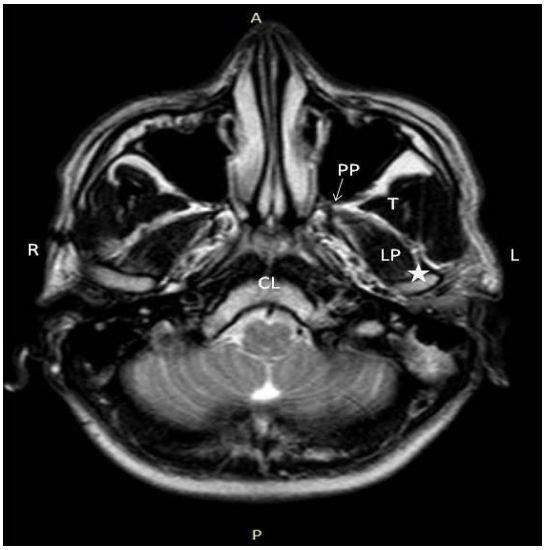

Fig 3. MRI scan of head region (T2 – axial image);
LP – Lateral pterygoid muscle;
PP – Pterygoid plate of Sphenoid bone;
CL – Clivus
☆ - Pterygoid fovea of the mandible

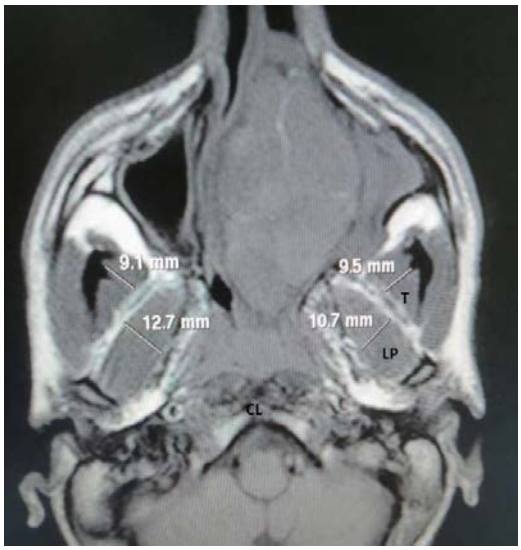

Fig 4. MRI scan of head region measuring thickness of -
LP – Lateral pterygoid muscle;
CL – Clivus

(II) Observations in MRI scan of adult patients

(a) Anatomical features – The lateral pterygoid muscle was present bilaterally in all of the MRI scans of the patients examined.

(i) Sites of attachment – In the present study, the lateral pterygoid muscle was found to attached anteriorly at the lateral pterygoid plate of sphenoid bone whereas, the posterior attachment of this muscle was found on the pterygoid fovea of the neck of the mandible. These attachments were observed in the axial images in T1 (Fig. 2) and T2 (Fig. 3) sequences. No unusual morphological variation was observed in any of the MRI cans of the patients examined.

(ii) Orientation of fibres – The fibres of the lateral pterygoid muscle were found to converge backwards and laterally in axial images.

(b) Morphometric evaluation – In the present investigation, the maximum thickness of the medial pterygoid muscle was evaluated at the level of the clivus. The mean thickness of the lateral pterygoid in the present study was 10.82 ± 1.50 cm with a range of 8.6 – 14.8 cm.

DISCUSSION

Knowledge of the topography of the lateral pterygoid muscles is essential for oral and maxillo-facial surgeons. The findings pertaining to the lateral pterygoid muscle were compared to the findings of the previous literature. In many aspects, the observations of the present study almost correspond with the earlier studies conducted on the lateral pterygoid muscles.

In the present study, the lateral pterygoid muscle was found to be present in all the cadaveric specimens and MRI scans of the adult patients examined. It was observed as a short thick muscle which comprised of two heads originating from the cranium – upper head and the lower head in all the specimens. Both the heads of the lateral pterygoid muscle were found to converge and were found to pass backwards and laterally. The mandibular attachment of the lateral pterygoid muscle was observed at the pterygoid fovea in front of the neck of the mandible. However, few of the upper fibres of the lateral pterygoid muscle were found to be attached at the articular disc and capsule of the temporo-mandibular joint in 24 specimens out of 30 specimens studied. However, in 6 of the specimens, it was noticed that all the fibres of the lateral pterygoid muscle were solely attached on to the neck of the mandible in the present investigation.

Similar findings regarding the attachment of the lateral pterygoid muscle have been reported by various researchers. Akita K et al noticed that most of the fibres from the upper head of the lateral pterygoid muscle were attached to the disc of the temporo-mandibular joint, whereas most of the fibres from the lower head of the lateral pterygoid muscle were attached into the neck of the condyle of the mandible [4]. Few fibres were seen to be attached into the area between the capsule of the temporo-mandibular joint and neck of the condyle of the mandible. They also suggested that the lateral

pteryoid muscle could not be divided into distinct parts as the upper and the lower heads were not distinguishable from each other near their insertion site. Naohara H et al described that the uppermost fibres of the lateral pterygoid muscle were inserted into the articular disc of the temporo-mandibular joint, while rest of the fibres were inserted into the pterygoid fovea of the neck of the mandible [5].

There are varied reports on the mandibular attachment of the lateral pterygoid muscle in the literature. Zhang L and co-workers reported that the fibres of the superior head of the lateral pterygoid muscle were inserted on to the condyle of mandible in 52 % , to the capsule of temporomandibular joint in 24 %, to the anterior attachment of the disc in 14 % and to the articular disc in 10 % of the specimens [6]. Naidoo LC reported that the upper head of the lateral pterygoid was attached to the capsule, meniscus and pterygoid fovea of the condyle in 65 %, to the condyle in 27.5 % and to the meniscus in 7.5 % of the cases. Earlier studies noted that 29.5 % of the fibres of the lateral pterygoid muscle were inserted into the meniscus of the temporo-mandibular joint through a tendon [7]. Heyling DJ et al reported that the superior head of the lateral pterygoid muscle was attached to the capsule of the temporo-mandibular joint and to the condyle of the mandible [8].

Bravetti P et al prepared blocks of temporo-mandibular joint and its surrounding muscles and sectioned them into different planes – sagittal, horizontal, oblique and frontal sections. The sagittal sections showed the insertion of the lateral pterygoid over the whole height of the neck of the mandible whereas the horizontal sections demonstrated its insertion over the whole width of the neck of the mandible. However, in frontal sections, the superior part of the lateral pterygoid was observed to be attached on the anterior border of the disc of the temporo-mandibular joint [9]. In another study, Velasco JRM et al described that the superior head of the lateral pterygoid muscle was inserted into the anteromedial two- third of the disc of the temporo-mandibular joint [10].

In the present study, lateral pterygoid muscle was present bilaterally in all of the MRI scans of the patients examined. It was found to be attached anteriorly at the greater wing and lateral pterygoid plate of the sphenoid bone whereas posterior attachment of this muscle was at the pterygoid fovea in front of the neck of the mandible in axial images. However, in an earlier investigation through an MRI study, the attachment of the lateral pterygoid muscle was categorized into two types – Type I, in which the fibres of the superior head of the lateral pterygoid muscle were attached to the disc of the temporo-mandibular joint and fibres of the inferior head of the lateral pterygoid muscle were attached to the condyle of the mandible, whereas in Type II, fibres of the superior head of the lateral pterygoid muscle were attached to the disc and condyle of the mandible and fibres of the inferior head of the lateral pterygoid muscle were attached to the condyle [11].

In another MRI study conducted by Mazza D et al, the insertion of the lateral pterygoid muscle was categorized into three types – Type A in which the single muscle bundle was inserted on to the capsule of the temporo-mandibular joint

Table 1. Number of heads of lateral pterygoid muscles

Study	Number of heads
Naohara H et al[62]	Two heads in 65% Three heads in 20% Single head in 15%
Abe S. et al[68]	Single head in 53 %
Gaudy JF[69]	Single head in all specimens
Fujita et al[70]	Three heads in all specimens
Present study	Two heads in all specimens

and condyle of the mandible. In type B, one muscle bundle was inserted on the disc and the other one on the condyle of the mandible, whereas in type C, a single muscle bundle was inserted only on the disc of the temporo-mandibular joint. They also considered that the type C might contribute to the dislocation of the disc as it was directly attached only on to the disc. No such differentiation of the insertion site of the lateral pterygoid muscle was observed in the present MRI investigation [12].

In the present study, it was found that the lateral pterygoid consisted of two heads – upper and the lower head. The observations of the present study almost correspond to the results of the earlier researchers. However, slight variations are reported regarding the number of heads of the lateral pterygoid muscle (Table 2). Naohara H et al classified the lateral pterygoid muscle into the three types depending upon their number of heads – 65% of their specimens possess two heads; 20% cases were seen to have three heads and single head was present in 15% cases [5]. Abe S et al found that the muscle consists of a single head in 53 % of the specimens studied [13]. Gaudy JF described that the lateral pterygoid muscle consists of a single head only [14]. Fujita S et al reported that the lateral pterygoid muscle consists of three

heads – upper head, lower head and inner head [15].

CONCLUSION

Precise knowledge of the normal anatomy and morphological variations of the lateral pterygoid muscles is relevant for dentists, maxillo-facial surgeons, radiologists and anatomists. The results of the present study highlight a variety of anatomical details of the lateral pterygoid muscles in cadaveric specimens especially in relation to the attachment of lateral pterygoid muscles to capsule of the temporomandibular joint. The discrepancies between observations of the present study and those of previous researchers could possibly be due to regional variations and racial differences.

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Case Report

DUAL BLOOD SUPPLY TO THE LEFT LOBE OF LIVER - AN EMBRYOLOGICAL AND CLINICAL SIGNIFICANCE OF ABERRANT ACCESSORY LEFT HEPATIC ARTERY

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ABSTRACT

Hepatectomy refers to the partial or complete removal of the liver. The coeliac trunk and its branches provide the major blood supply to the liver and hence, the knowledge about the coeliac trunk branches, their orientation and their variations become critical not only in deciding the procedure of hepatic surgeries but also influencing the outcome of the surgery. The present case report presents an observation of an aberrant accessory left hepatic artery during the routine dissection for undergraduate students at Lady Hardinge Medical College, New Delhi. The practice of viewing this variation on an embryological and molecular basis would prove to be beneficial not only to correlate liver function with the variant anatomy but also in planning a targeted procedure in cases of whole or split liver transplant surgeries.

Keywords: Looping, Internal carotid artery, Variations

INTRODUCTION

The evaluation of the coeliac trunk variations, its branches supplying the liver is of utmost importance as it helps in retaining greater amount of liver during hepatectomy. The variations are important to be angiographically demonstrated which influences the outcome of surgical and interventional radiological procedures [1]. The presence of aberrant hepatic artery; whether replacing or accessory is often reported in patients with gastric cancer and is known to cause post-operative liver dysfunction after its resection [2]. Awareness of anatomical variations of the hepatic arterial system also proves its significance in various hepatobiliary surgical procedures.

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CASE REPORT

The coeliac trunk gave out three branches, the common hepatic, splenic and left gastric artery. The common hepatic artery further divided into right and left hepatic arteries supplying the respective lobes. The left gastric artery was observed coursing upward and to the left towards the cardiac end of the stomach where it turned sharply and followed the lesser curvature of the stomach. It divided into an anterior and a posterior branch supplying the corresponding surfaces of the stomach (Fig. 1, labelled 2, 2B). A long anterior left hepatic artery (ALHA) (Fig. 1, labelled 2A) arising from the apogee of the left gastric artery measuring 44.08 mm was observed supplying the left lobe of liver through the vertical limb of the lesser omentum in the fissure for ligamentum venosum. A branch arising from it, 22 mm in length supplying the cardiac end of the stomach was also observed (Fig. 2). The above said liver also exhibited an incomplete fissure for the ligamentum teres.

DISCUSSION

In the present case, we report three extrahepatic arteries in a human cadaver. This anomaly is the first observation among 24 human adult specimens from a 3-year retrospective screening of the dissection records at our institute. The accessory left hepatic artery in our case was supplying the medial inferior area (Fig. 3) of the hepatic artery segmentation [3] signifying that the segment is having dual blood supply. According to Couinaud's classification [4] it belonged to segment II. The present variation should be defined as type 5 in Michel's [5] and type 2 in Hiatt's classification [6] of hepatic arterial system. In our case, the ALHA is observed to supply the cardiac end of the stomach which is not mentioned in any of the classifications.

Embryological and phylogenetic basis

Unlike the norm of the vasculogenesis of the body, the aortae and its system of branches follows an extended unbranched course until it supplies the capillary plexuses which sprout from the intersegmental points. These points are diligently followed by the dorsal branches unlike the ventral and lateral segmental arteries which are often found arising at more frequent intervals from the dorsal aorta. The segmental influence on the ventral and lateral plexuses are evident, but its expression is imperfect [7] hence abnormalities in the ventral branches of the aorta is commonly observed compared to dorsal and lateral branches.

The presence of the ALHA in our case is perhaps due to persistence of one of the ventral segments which failed to adhere the segmental alignment followed normally. The ventral and dorsal branches grow out completely before the fusion of the aortae. The ventral pair fuses to produce a single median stem by the time the embryo attains a length of 5 mm [8]. The fusion initially takes place in the middle of the unpaired aorta and proceeds cranially and caudally from this point in a similar fashion as the central nervous system in the developing embryo [7]. The coeliac trunk arises by the seventh and the eighth ventral segments. The artery wanders

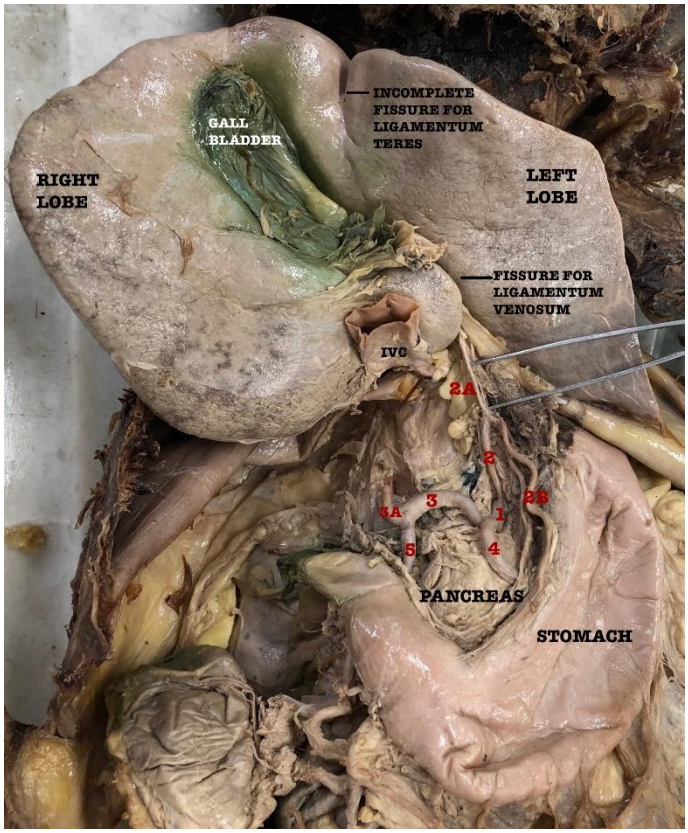

Fig. 1: Liver reflected cranially showing the visceral surface. 1) coeliac trunk 2) left gastric artery 2A) accessory left hepatic artery 2B) anterior and posterior branches of left gastric artery 3) common hepatic artery 3A) right and left hepatic artery 4) splenic artery 5) gastro- duodenal artery (IVC: inferior vena cava)

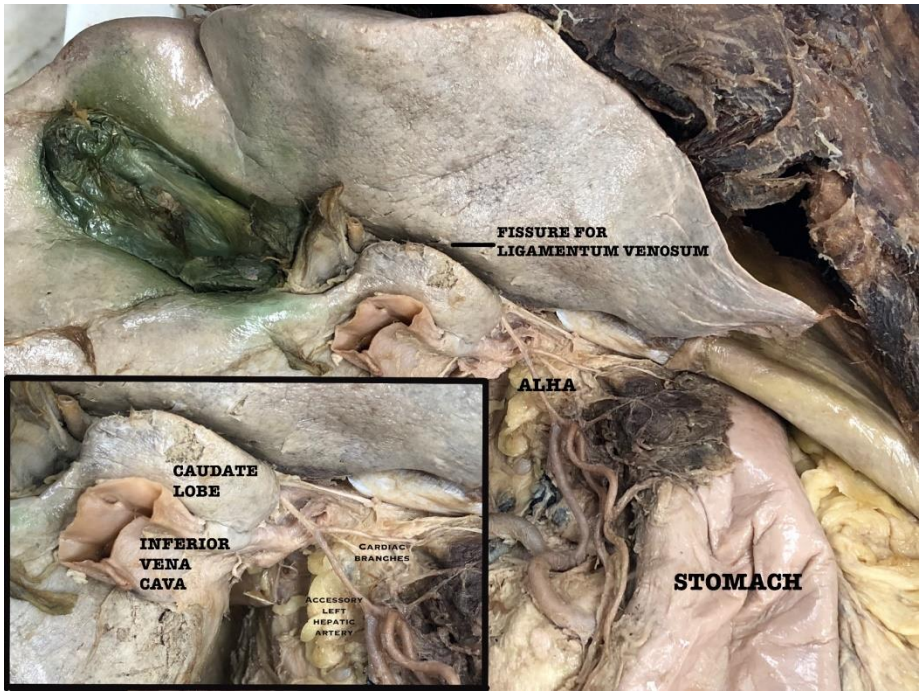

Fig. 2. ALHA (accessory left hepatic artery) supplying the left lobe of liver passing in the vertical limb of the ligamentum venosum. Inset showing cardiac branch arising from ALHA

[9] from the seventh cervical to the twelfth thoracic segment and this displacement of eleven segments is due to the proportionate dislocation of the upper part of digestive tract. Theories for the exact manner of the arterial displacement have been put forth [10].

1. Intersegmental anastomosis: Initially, numerous anastomoses connecting the ventral vessels exist but as the embryo develops, not a single other vessel exists between the points of origin of the three chief ventral vessels.

2. The presence of a non-segmental root of origin of the ventral branches of the aorta and its anastomoses between the wandering arteries and the aortic wall, with the ensuing atrophy of the older roots. The original roots being segmental, any non-segmental position of the vessel is explained by the acquirement of secondary non segmental roots.

Therefore, in view of the above points, the presence of the ALHA is due to the persistence of a non-segmental ventral branch from the dorsal aorta supplying the liver, and its segmental anastomosis with the gastric sinistra, (Fig. 4) a branch of the coeliac trunk as the latter wanders caudally to reach its adult level at twelfth thoracic segment. The presence of the cardio-esophageal branches from the ALHA in our case is further justified by the presence of oesophageal rami (Fig. 4) from arteria gastric sinistra.

It is further reinforced by the fact that the arterial supply to the liver in early gestational age is from three main sources: 1. The left hepatic artery from left gastric artery 2. Middle (common hepatic artery from coeliac trunk and 3. Right hepatic artery from superior mesenteric artery. Eventually the right and left hepatic arteries get atrophied and common hepatic artery supplies the whole liver. This adult pattern is exhibited in 67% of individuals and persistence of fetal pattern results in anatomical variations of liver vascularity [5,11,12]
It can be further postulated that the persistence of the fetal pattern took place in our case due to the incomplete fissure for the ligamentum teres hepatis, demanding surplus blood supply to the left lobe of liver.

Molecular Basis

The initial configuration of the circulatory system of the embryo includes [13]:

1. Continued formation, migration and coalescence of endothelial precursor cells (EPCs)
2. Angiogenesis
3. Vascular intussusception (non-sprouting angiogenesis) - existing vessel splits to generate additional vessels.
4. Intercalation of new EPCs into the walls of existing vessels

Vascular endothelial growth factors (VEGFs) and their receptors play a pivotal role in mediating angiogenesis while Notch proteins (signaling) have a role in cell fate decision by remodeling the primary capillary plexus. TIE (tyrosine kinase with Immunoglobulin-like and EGF-like domains) receptor groups act in parallel to promote angiogenesis as well. TIE2 and Ang1 are involved in regulating intussusception of the vasculature. Ang2 under

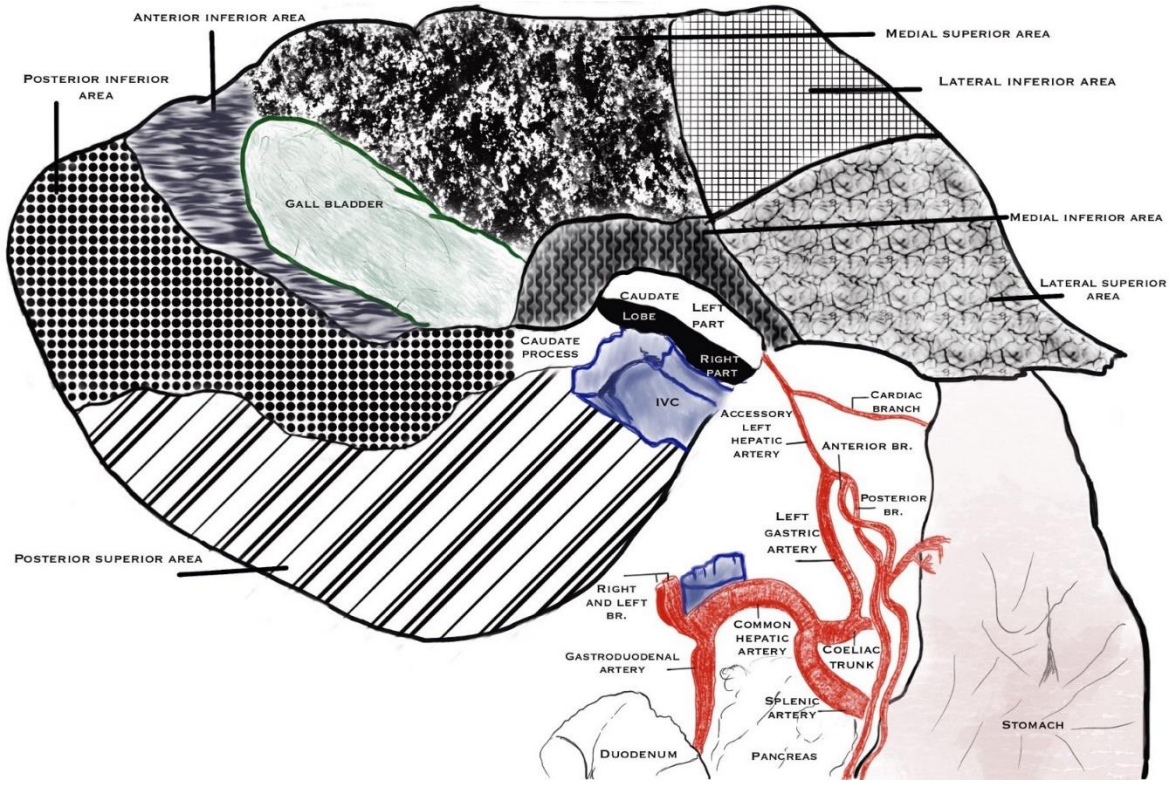

Fig. 3. Diagrammatic representation of the specimen and illustration of the segmentation of the visceral surface of the liver based upon the principal divisions of the hepatic artery and accompanying hepatic ducts (3)

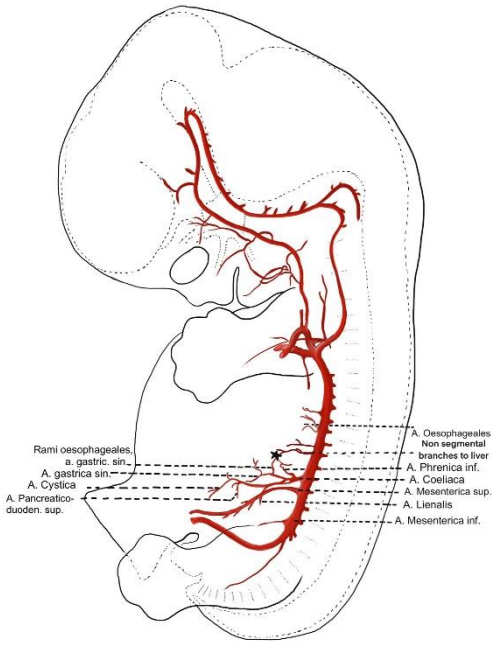

Fig. 4: Graphic reconstruction of the arterial system of a human embryo 15.5 mm long, Modified from Keibel and Mall (10). Black star marked showing anastomosis between gastrica sinistra and non-segmental branches to liver

the stimulatory effect of VEGF stimulates sprouting. The degree of vascularity either by formation or pruning of the vessels may be mediated by oxygen dependent regulation of VEGF and nitric oxide levels through counteracting hypoxia by stabilization of the transcription factor HIF1α (hypoxia inducible factor1α).

Defect in Notch protein signaling could have resulted in the defect in remodeling of the primary capillary plexus of the ventral branches of the aorta (segmental as well as non-segmental) leading to persistent anastomosis between gastrica sinistra and non-segmental branch supplying the liver. Perhaps 'abnormal intussusception' of the vasculature by TIE and Ang1 may have resulted in the splitting of the gastrica sinistra into left accessory hepatic artery with the former continuing as left gastric artery. Failure of the pruning of the vessels due to unbalanced regulation of the oxygen dependent VEGF and Nitric oxide (NO) may have caused the persistence of the said artery as well.

In accordance with the dictum: ontogeny recapitulates phylogeny, the origin of the accessory left hepatic artery from left gastric artery and supply of the stomach via the accessory artery is probably due to the fact that the ruminants' stomach is supplied by various arteries including hepatic artery, left gastric artery, right and left ruminal arteries which are the branches of the coeliac artery [14].

Clinical significance

The ALHA in the present study is present in the lesser omentum which poses a risk of liver parenchyma necrosis by surgeons approaching the gastroesophageal junction for stomach mobilization by dividing the lesser omentum. Knowledge of these variations is important in planning whole and split liver transplantation surgeries, as livers with multiple vascular pedicles pose a challenge during anastomosis.

The development of the arteries of the gut has been elaborated and explained upon but from the standpoint of the embryological and molecular basis due to which the variations exist has not been assigned. It has not been clearly discussed in the previous literature nor has the cause of the variations been explained. The present case hopes to create a much holistic approach of viewing such variations.

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Guest Submission

MEDICAL PROFESSIONALS UNDER CONSUMER PROTECTION ACT, 2019 - AN ANALYSIS

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ABSTRACT

Negligence plays a pivotal role in law. The law of wrongs, popularly known as the law of torts is based on negligence. It was in 1986 when for the first time one such tort of negligence was covered through Consumer Protection Act (COPRA) to protect the rights of innocent consumers at large. However, in the statute the "health care" amenities were not in the preview of the act, but it was grasped though judicial pronouncements and since then healthcare was treated as a service under the act. After about 33 years of the enactment of COPRA, 1986, was replaced with Consumer Protection Act, 2019. When the bill for the Consumer Protection Act was placed before the houses of the parliament it created ruckus in the medical fraternity as in the bill the term "health care" was specifically included in the ambit of services. The doctors showed speculative apprehension of its mishandling which could undermine the entire community. This in turn forced the legislatures to drop the same from the bill. Therefore, this article is an attempt to compare the application of the acts. The scope of the comparison will remain limited to health care workers. The article focuses on real time execution of the statute and an effort for striking a balance between the liabilities of a doctor in case of any negligence in general and preventing health care workers from unnecessary accusations in particular.

Keywords: Consumer Protection Act, Health Care/Medical Professionals, Negligence, Liability

INTRODUCTION

"Health is a precondition for life and life is a precondition for ecological balance in whole".

In 2021 health and healthcare issues have raised the alarm in the mind of the public at large in general and medical professionals in particular. This fact cannot be denied that healthcare is one of the major state concerns where medical professionals including para-medical and other healthcare support staff is/are in need of hustle free work environment.

A medical profession can be defined as any person registered, licensed or certified to provide health care services to a natural person

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Meaning thereby, medical professionals include physicians, cardiologists, clinical dieticians, psychologists and other wide range of professionals in process of rendering health care services, which was eventually covered as "services" under Consumer Protection Act, 1986. Whereas healthcare services are broadly welfare services for noble social cause to heal the human soul from the emotional, psychological and physical stress and pain of a patient along with near and dear ones. This was in 1995,[1] when healthcare has been captured by the interpreters of the Indian constitution and kept it in legal claw under which medical professionals' moral obligation was left no more moral but totally legal where healthcare has been attached with services.

Though, controversies and criticism were on the top, but no voice was sturdy enough and therefore, healthcare continued to be a deemed service under Consumer Protection Act, 1986, and dedicated healthcare professionals faced incongruity of the system. At the same time, it cannot be denied that due diligence is the core of medical profession and negligence may lead to fatal consequences. Few illustrations are there, where gross negligence in medical profession can be seen as well ashaming the noble profession. But before putting the burden on health care worker under the act, one has to ascertain that there is a gross negligence in contrast to the efforts with due diligence, which subsequently meet failure. The efforts /labours of majority of medical professionals cannot be on stake because of handful of mal practitioners and marginalised defaulters in the medical profession. Undoubtedly, an effort may raise an error, but the question is, whether the efforts of the saviour of mankind are to be protected or they shall be backed by the legal sanction or (how legal sanction can be questioned, it has to be followed) coercive methods for no reason.

Recently, the Consumer Protection Bill, 2018, has been signed by the competent authority. It is essential to highlight that initially while introduction of the bill in the house, the word "healthcare" was given place in the list of services and the same was passed from the Lok Sabha. But during discussion and debates in the Rajya Sabha due to uninterrupted demands and presentations from various medical organisations and health care service providers the word "healthcare" was dropped out from the list of services. However, the language of the bill was amended before its enactment and the word 'health care services' was removed from the final definition of the services. The original bill defines "Services" under Section 2(42) which reads as-

"…service of any description which is made available to potential users and includes, the provision of facilities in connection with banking, healthcare, boarding or lodging or both…but does not include the rendering of any service free of charge or under a contract of personal service …"[2]

BACKGROUND

Medical profession is one of the oldest professions in the world typically with in reference to with that of Indian culture, where doctors are given the place of God. However, with the advent of time, society had witnessed certain malpractices being carried out in medical profession as well. Therefore, application of certain degree of regulation and reform become essential to safeguard the interest of the people.

For long time, people did not have an appropriate body to adjudicate the issue as per their complaints. This was only after the amendment Act of 1964 (IMC Act 1956), that the Indian Medical Council Act, highlighted the regulations to address such problems and issues. Therein, the misconduct of a medical professional can be punished by suspension or deletion from the roll.

However, it came to light that this action was insufficient as it does not have desired deterrent effect. Also, impartiality of the council was questioned along with the fact that there lies no power to grant compensation to the injured patient. The relief by recourse to various criminal and civil remedy were available but they had a low success rate due to prolonged hearings and rigidness of procedure.

The Consumer Protection Act, 1986 came as a survivor to many aggrieved patients by creating consumer dispute redressal agencies, popularly referred to as C.D.R.As. However, as speculated, the applicability of the act was challenged by the doctors mainly on the grounds of the kind of relationship shared by the doctors and the patients who rested more on trust and faith rather than a trading service aiming solely at profit.

HEALTH CARE FACILITIES & SERVICES UNDER THE ACT OF 1986 ACT :

In the case of Indian Medical Association v. V.P. Shantha & Others,[1] the three-judge bench of Hon'ble Supreme Court held that medical practitioner who gives services to a patient in the nature of patient-doctors consultation, analysis-finding and conclusion on the basis of diagnosis to treat the patient, would surely come within the ambit of the term 'services' by virtue of Section 2(1) [3] (o) of the Consumer Protection Act, 1986. It is peculiar to note that to come within the ambit of the term 'services' by virtue of Section 2(1) [4] (o) of the Consumer Protection Act, 1986, it has to be a "contract for service" and not "contract of service". The conceptual difference between the two is that the former denotes a contract where one party provides services to the another, eg., a professional or technical service, the latter is a contract in the nature of a master and servant relationship.

Whether "Free" Medical Services are covered Under Consumer Protection Act:

The Apex Court in, the case,[5] formulated a shrunken adaptation to demarcate as to what will comprise in 'free medical care'. It segregated the services in general like offering free of cost to everyone & the services availed on payment; and services for which fee is charged but are made available free of cost to the persons who cannot meet the expense.
The Court held that the services provided against payment, along with services to those who cannot afford but are not free in nature, are the subject matter of this act and none other.

WHAT CONSTITUTES MEDICAL NEGLIGENCE?

Literally speaking, negligence means failure to take proper care than as expected. Winfield and Jolowicz in one of the writings clarified that due

care is the responsibility of every service provider and this is backed by the legal obligation. When there is a lapse of the legal duty to take care which results in damages is called negligence. Winfield himself classified negligence as a tort which may have the right to initiate legal action only when there is a breach of legal duty to take care along with damages to the plaintiff.[6]

In Poonam Verma v. Ashwin Patel & Ors.,[7] this was decided by the hon'ble Apex Court that the word negligence has many expressions and may have different implications, it may be direct or indirect, deliberate or through casual approach, vigorous, collateral, relative, absolute, contemporaneous, criminal/hazardous where all are neither will full nor punishable. Only wilful or reckless negligence are the Negligence per se."[8]

In Jacob Mathew v. State of Punjab & Another [9] the Hon'ble Apex Court observed,

"Any sensible person inflowing into a business or occupation or profession….to be called a professional of that discipline, impliedly affirms the person dealing with him that the minimum required skills of the profession and for which he professes to possess shall be employed and such deployment of the sills will be furthermore with the utmost good faith inclined with reasonable degree of care and caution……the only guarantee which such a specialized one can give…that he is holds the minimum requisite skill of that branch of practice in which he is engaged and while undertaking the performance of the task entrusted to him he would be exercising his skill with reasonable competence."[10]

To establish a case of negligence following elements have to be fulfilled:

1. Duty of Care to the plaintiff,
2. Breach of Duty,
3. Damage.

STANDARD OF CARE:

In the leading English case under law of tort, Bolam v.Friern Hospital Management Committee,[11] certain rules were laid down for those cases that involved skilled professionals such as doctors so as to determine the reasonable care in cases involving negligence. This rule, popularly known as Bolam test, states that if a skilled professional reaches the standard of care and diligence, they are not acknowledged to be negligent. However, Bolam test was discarded in the 2015 Supreme Court decision of Montgomery v. Lanarkshire Health Board.[12]

In Indian perspective, the principle of 'Standard of care' was laid down by the Hon'ble Supreme Court in the case of Dr. Laxman Balakrishna Joshi v. Dr. Trimbark Babu Godbole [13] and A.S Mittal v. State of U.P.[14]

DRAWBACKS OF CONSUMER PROTECTION ACT, 1986:

Before the enactment of COPRA, 1986 there were certain enactments providing relief to the consumers indirectly, such as Food Adulteration Act, Essential Commodities Act, etc. However, relief granted under these statutes were only in the nature of punishment provided to the accused, but no direct benefits were granted to the victim/consumer. Hence COPRA, 1986 emerged as a blessing to various consumers of goods and services that

provided compensatory relief. Nevertheless, still there were many inadequacies attached to it, such as, the 'services' for which some fee is charged was the only subject matter of dispute under this Act. The doctors who rendered services free of cost and the hospitals where services are given free of cost are not within the realm of this act. Also, the act does not vest the Consumer Redressal Forum with the power to entertain the cases suo-moto. Moreover, the major problem that lies behind the operation of the act is the uncertainty and default in execution of the orders passed by the Consumer Court. One such major loophole lies in the fact that all the rights of the consumers in the act, talk about the right to choose, right to safety etc., which do not appear justifiable thus degrading the very spirit of the act. The act also failed to include services through e-commerce in its dealings.

COPRA 1986 v. 2019: A COMPARITIVE ANALYSIS:

KEY POINTS	OLD ACT	NEW ACT
Domain	Goods and Services for Consideration. (except free and personal services)	All goods and services, (including telecom and housing construction), and all modes of transactions such as online, teleshopping, etc., for consideration. Free and personal services are still excluded.
Pecuniary Jurisdiction	District forum (upto 20 lacs) State commission (from 20 lacs to 1 crore) National commission (from 1 crore and above)	District forum (upto 1 crore) State commission (from 1 crore to 10 crore) National commission (from 10 crore and above)
Territorial Jurisdiction	Where seller has office	Where complainant resides or work.
Regulatory Body	No Provision	Central Consumer Protection Authority to be formed.
Mediation[15]	No Provision	Court can refer for mediation under Section 80.
Price to decide Jurisdiction	Earlier MRP	Now discounted or actual price.
E-Commerce	No Provision	All provision applicable to direct sellers to extend to E-Commerce as well.
Penalties	If a person does not comply with orders of the Commissions, he may face imprisonment between one month and three years or fine between Rs 2,000 to Rs 10,000, or both.	If a person does not comply with orders of the Commissions, he may face imprisonment up to three years, or a fine not less than Rs 25,000 extendable to Rs one lakh, or both.

CONCLUSION

The fact, that the doctors are the savoir and the hope of this world cannot be marginalised, especially when a new disease or health problem occurs, and medical science is silent on the issue. Under such circumstances doctor can neither say no nor can he affirm that he has the capabilities and specialities to treat the issue. Prevalent Covid-19 pandemic may be considered one of the examples of the same. Here medical professionals have to work on the basis of the experiences and probability. Though the maxim of probability has no space in the maxim of due care or in term of holding the minimum level of required skills. This may lead to two opposite positions either to say no to treat or to make efforts in good faith. Furthermore, this cannot be denied that present advancement and development of the medical science and research is also based on the idea of the experiences and probability. Here in present legal system if health care professional will be kept under the clutches of law, this will lead to wholesome unforeseen adverse effect.

Most of the countries possess various penal provisions in the domestic criminal jurisprudence to treat the issue if doctor is running beyond call and deliberately or carelessly treats the patient. The same has also been highlighted in the case of Jacob Mathew v. State of Punjab,[16] the Court looked upon to decide the issues concerning criminal negligence of doctors under the Indian Penal Code. The court added that Bolam case[17] principle should not be generalised. Even after the cautiousness expressed by the Hon'ble Supreme Court of India, false and frivolous cases are there against the doctors and it cannot be denied that most of them are to extract the money from them or to flee from the liability of payment due to the hospital and doctors. This is also evident that in case of causality in the hospital, it becomes tough for health professionals and health care centres to save themselves from the violence caused by the caretakers of the patient. In such a situation the procedure of the criminal liability as explicitly elaborated by the court should be that the identification of the doctor responsible for lack of due care or as a prime suspect, should be done by referring such case to a senior government doctor of the same speciality or to the committee of doctors who would ascertain the correctness of the alleged lapses. Thereafter, only upon confirmation of negligence, a notice be issued against the concerned doctor.

However, there are many cases where the inclusion of medical services under the profound umbrella of "services" has proved to be a bane to the medical professionals

Due diligence is core and will remain so in the healthcare establishment. However, it is not easy to establish "lack of due diligence" for the petitioner or the applicant. Similarly, it is hard on the part of the medical professional to establish the presence of utmost due diligence. There is one inseparable line between success and failure of the case of critical care. Viewpoint of a patient and the caretaker of the patients has remained biased ever since and when healthcare professionals got captured in the clutches of the Consumer Protection Act 1986, this issue became furious and somehow, it turned out to be an act of blackmailing and money-making task for many. It is true to say that this is definitely, the misuse which was not

app rehended earlier but even after two decades of the 21st century when now medical professionals are working for various commercial gains, more specifically when healthcare services are not only technology based but also expensive beyond the common reach.

But at the same time, it is essential to keep an eye upon the malpractices gradually getting rooted in the medical sector thus resulting in escalating cases of medical negligence due to the mushrooming of the hospitals and diagnosing centres. It is evident from daily newspapers highlighting outrageous news of gross medical negligence such as doctors leaving behind surgical equipment inside the body of the patient, causing serious injuries and health issues, sometimes even resulting in the death of the patient.[18]

Therefore, the specific technical addition or deletion of the 'healthcare' under the ambit of "Services" either in COPRA 1986 or 2019 would not do any good to regulate the current scenario. A balance needs to be maintained to assure that doctors are not baselessly exploited or annoyed for any mishap considering the dynamic nature of the profession, but undoubtedly, any act of practitioners deviating from due diligence and standard of care cannot go unpunished. As it is an established principle that "he who seeks equity, must do equity".

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Original Article – Student Authors

DERMATOGLYPHICS-A Predictor of Disease

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ABSTRACT

Dermatoglyphics is the scientific study of pattern of dermal ridges on the palmar surface of digits, palm and sole. Characteristically, hair do not grow in this area. These ridges serve well to enhance contact. The development of these ridges and the development of nervous system occur simultaneously in the intrauterine period. The pattern of dermal ridges begins to develop around 13th week and is completed by 19th week of intrauterine life. Once the finger-print pattern develops, it does not change and persists throughout life. Unusual dermatoglyphic patterns often relate to genetic disorders. Dermatoglyphics may be used as an additional screening tool to identify early risk factors that may help prevent additional complications of various diseases. In this review, we will be discussing Dermatoglyphics and its important role in the diagnosis of diseases which have some genetic basis.

Keywords: Dermatoglyphics, dermal ridges, fingerprint.

INTRODUCTION

The word dermatoglyphics is derived from two Greek words (derma - skin and glyphe – curve). The term "dermatoglyphics" was coined by Dr. Harold Cummins in 1926 [1]. The importance of dermatoglyphics lies in the morphological constancy of the dermal ridge arrangements from the time of formation until death. The dermal ridges are fully formed by the fourteenth week of foetal development. Therefore, only genetic and prenatal environmental factors can influence ridge formation [2]. These dermal ridges play a very crucial and important role in the personal identification of an individual. For example, the dermal ridges are important for forensic purposes, in twin diagnosis, racial variation and have applied values in various diseases and syndromes [3]. Since each person's fingerprints are unique, we can understand one's innate potential, personality and preferences by analysing dermatoglyphics [4].

Dermatoglyphics refers to the scientific study of the skin ridge patterns on the fingers, toes, palm and sole. The purpose of these ridges is to impart firmer grip and avoid slippage [5].

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Dermatoglyphic patterns have polygenic inheritance and are affected by environmental factors. There exists relationship between epidermal ridge and fetal volar pads, because in course of development the ridge pattern is formed at the site of these pads [6]. During development various creases develop on the brain and are reflected on the fingerprints representing the various regions of the skin and brain developing from the same ectoderm. It is probable that an insult causing damage to one of these systems would damage the other. The environmental factors such as external pressure on fetal pads and embryonic fetal finger movements could influence ridge formation [7].

Papillary ridges are confined to the palm and soles and the flexor surface of the digits. The apertures of sweat glands open at regular intervals along the summit of each ridge. The epidermal ridges correspond to an underlying interlocking pattern of dermal papillae, an arrangement which helps to anchor the two layers firmly together. The pattern of dermal papillae determines the early development of the epidermal ridges [8].

The ridges once formed remain permanent and never change throughout life except in dimensions in proportion to the growth of an individual. The original ridge characteristics are not disturbed unless the skin is damaged to the depth of 1 mm. Ridges are the areas which decompose the last after a person dies. The ridge pattern can be affected by certain abnormalities of early development, including genetic disorders such as Down syndrome, and skeletal malformations such as polydactyly.

Absence of epidermal ridges is extremely rare. Functionally, epidermal ridges increase the gripping ability of hands and feet, preventing slipping [9]. Today, considerable progression has been made in understanding the association between dermatoglyphics and various medical disorders. Dermatoglyphic analysis has been investigated as a useful diagnostic and research tool in medicine and provides valuable insight on the inheritance and / or embryological basis of many known clinical disorders.

This review deals with dermatoglyphic studies mainly based on ridge patterns of palms and fingers and the pathologies related to it. Fingerprint ridge patterns can be separated into three major types: arches (5%), loops (70%), and whorls (25%) [9]. Though there are more than 100 ridge characteristics, called Galton's details in a single rolled finger print. (Fig. 1)

FINGERPRINTS AND CLASSIFICATION [10]

ARCHES:

The arches are the simplest and least frequent pattern, which pass across the finger with slight bow distally. They may be subclassified as plain or tented.

PLAIN ARCH - In plain arches the ridges enter on one side of the impression and flow or tend to flow out the other side with a rise or wave in the center.

TENTED ARCH - They are similar to plain arches with exception that the ridges in the

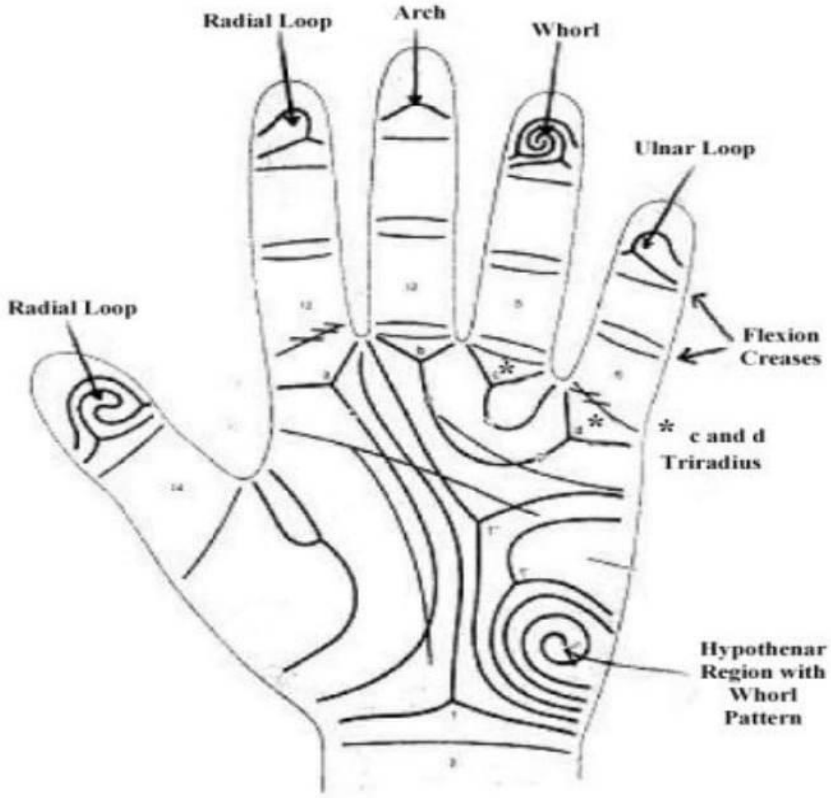

Fig. 1. Fingerprint patterns on the palm.

center form a definite angle; or one or more ridges in the center form an upthrust.

LOOPS:

The loop pattern has a triradius and a core. A triradius is a point at which three groups of ridges coming from three directions meet at an angle of about 120 degrees. The core is essentially a ridge that is surrounded by field of ridges, which turns back on themselves at 180 degrees. Loop can be either ulnar or radial.

ULNAR LOOP – The pattern in which the loops flow in the direction of the little finger.

RADIAL LOOP - In this pattern the loops flow in the direction of thumbs.

WHORLS:

The ridge courses follow circuits around the core in a whorl pattern.

ACCIDENTAL WHORL – It is the pattern with two or more deltas or a combination of two or more different types of patterns.

PLAIN WHORL - This pattern has two deltas and at least one ridge.

CENTRAL POCKET LOOP WHORL - This pattern consists of one or more recurving ridges.

DOUBLE LOOP WHORL – It consists of two separate loop formations.

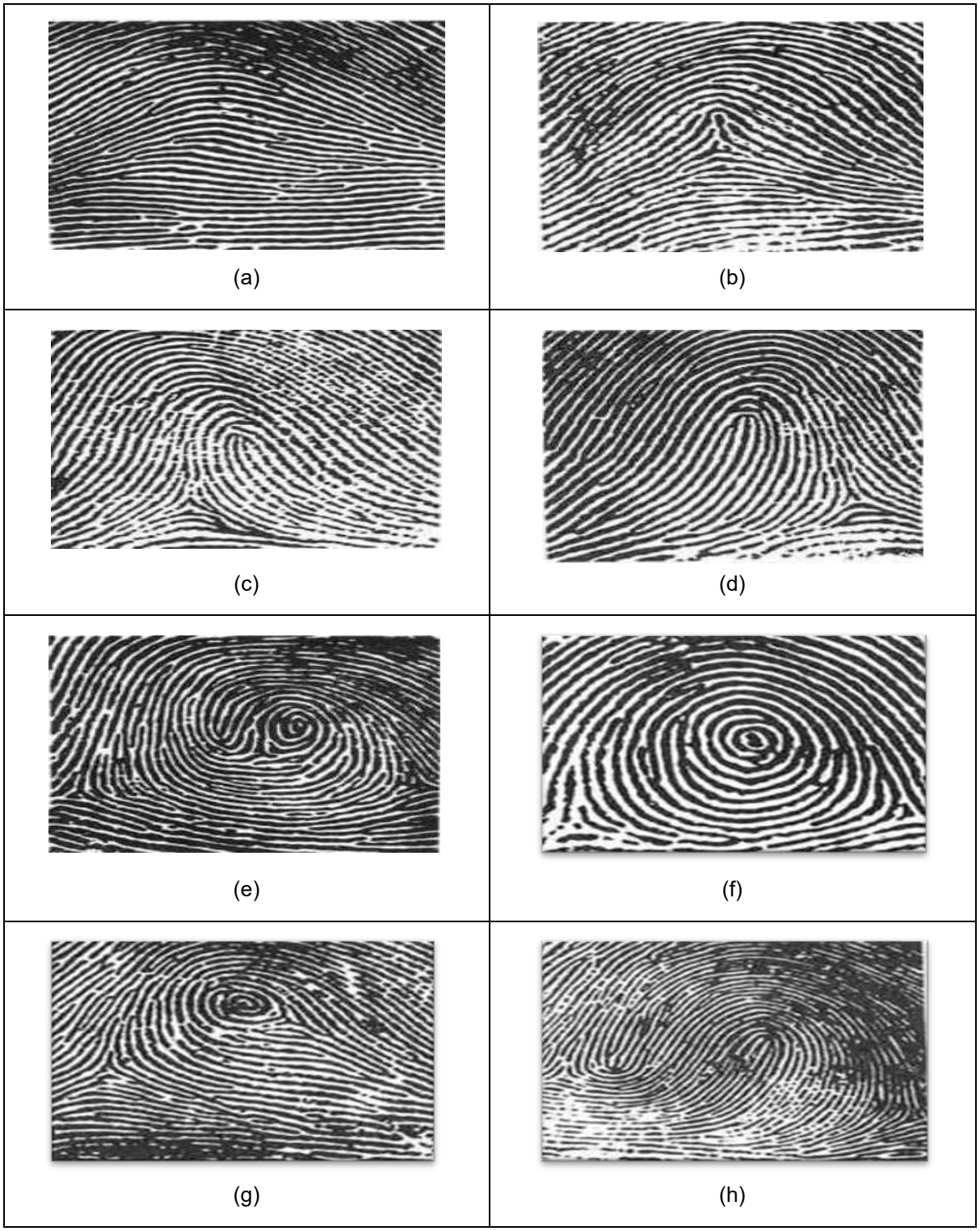

Fig. 2. (a) Plain arch; (b) Tented arch; (c) Ulnar loop; (d) Radial loop; (e) Accidental whorl; (f) Plain whorl; (g) Central pocket loop whorl; (h) Double loop whorl.

DERMATOGLYPHICS AND VARIOUS DISEASES

The development of these dermal ridges has been found to be affected by genetic and environmental factors during the developmental stages. Dermatoglyphics has, therefore, been accepted as a simple and inexpensive method for deciding whether a patient would have a particular genetic disorder or, not and any chromosomal defect [11]. Left handedness is the dominance of left hand over right hand. It is developed paternally and depends on which hand of the foetus is close to the mouth. Left handers have potentially a higher risk of developing schizophrenia. Left handers have more radial loop, modified radial loop and tented arch and decreased central pocket whorl, double loop whorl and simple arch as compared to right handers [12].

The left index ridge counts and fluctuating asymmetry in schizophrenic patients are different from those of the normal population [13]. This difference may serve as a diagnostic biological marker for screening people susceptible to schizophrenia. In both sexes, loops are more frequent in mentally diseased patients. Typographic diversity in fingerprint papillary patterns is more pronounced in males, and the differences between controls and cases show a tendency of significance. The bilateral comparison reveals a higher level of mismatching in fingerprint patterns on the second finger of both sexes in cases, and also on the third finger, but only in mentally diseased females [14].

The chromosomal anomalies such as the trisomies 13–15 (Patau's syndrome), 18 (Edwards' syndrome), 21 (Down's syndrome) and the sex chromosomes (Turner's syndrome X0 and Kleinfelter's syndrome 47, XXY) and deletion of the short arm of chromosome 5 (Cri du Chat syndrome) are recognized as having abnormal dermatoglyphic patterns [15].

The subjects with Downs syndrome show single transverse palmer crease (simian crease), bilateral radial loops on digits 4 and 5 and predominance of ulnar loops [16]. Dermatoglyphics and the analyses carried out have proved that they are invaluable in their clinical value, in selecting patients of Down's syndrome for cytogenetic analysis.

Predominance of ulnar loops over other patterns is also recorded in turner patients. Mean total finger ridge count in Turner syndrome remained higher than the normal females. The c-d interdigital ridge count in turners remained significantly ($p \leq 0.05$) higher than their normal female counterparts. Occurrence of whorls and arches in hypothenar region of 12% and 4% was respectively noticed in right palm of patients [17].

Type 1 diabetics show characteristic reduction in loops and notable increase in whorls and arches. Type 2 diabetics have increase in the frequency of whorls and decrease in ulnar loops. Males have a significant reduction in arches in right hand whereas females in left [18].

In subjects with essential hypertension, there is increase in Total Finger Ridge Count and Decrease in 'atd' angle [19]. In Bronchial asthma there is decreased number of arches, increased number of ulnar loops [20] whereas in tuberculosis predominance of whorls, decrease in loop pattern, High Total Finger Ridge Count, Narrower 'atd' angle [21] have been noticed. Carcinoma breast patients have reported a relatively high increase in the arches as compared to that in the controls [22]. In subjects with rheumatoid arthritis there is an increase in arches, decrease in whorls and loops in males and increase in whorls, decrease in loops on the 1st finger of both hands, increase in arches of 3rd digit and whorls on 4th digit of left hand in females [23].

CONCLUSION

This review brings out the importance of dermatoglyphic studies in various fields. The dermatoglyphics are important in forensic sciences due to their important feature that fingerprints are unchanged in due course even after death. By analysing these patterns, the analysers were able to find significant variations which represent those pathologies. Thus, we see that dermatoglyphics is a simple, inexpensive and bedside diagnostic aid for conditions of chromosomal aberrations and various inheritable diseases. The relevance of dermatoglyphics is not to diagnose, but to prevent by predicting a disease; not by defining an existing disease, but to identify people with the genetic predisposition to develop certain diseases.

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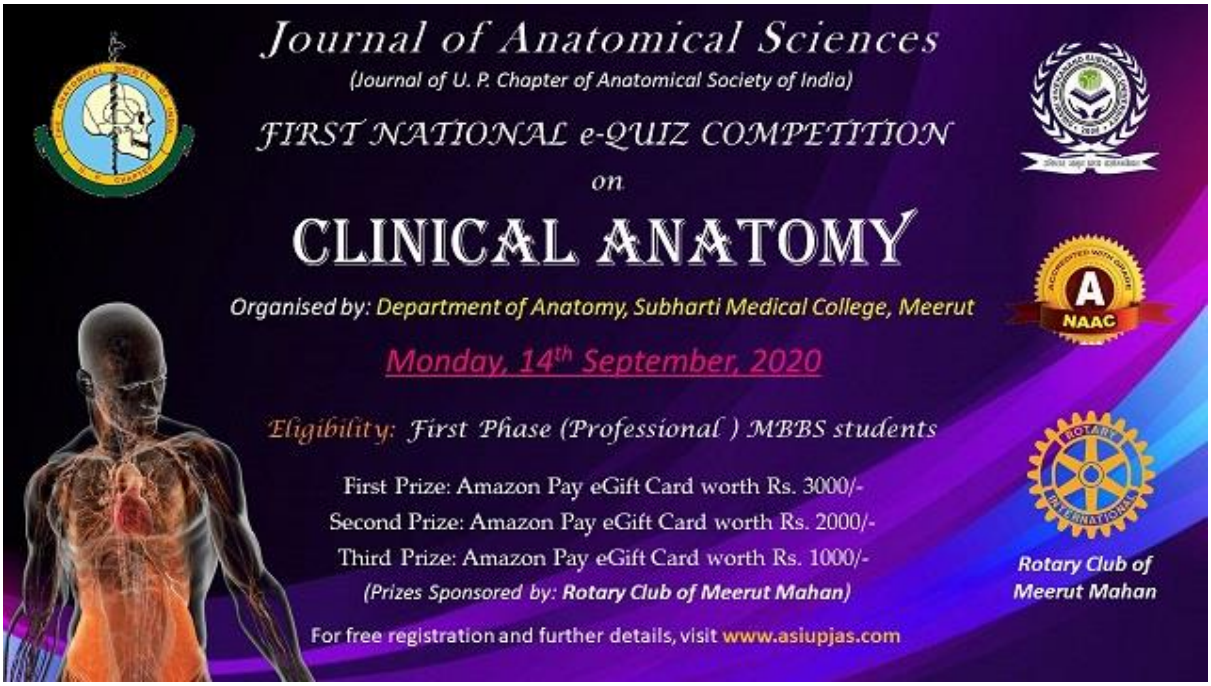

FIRST NATIONAL E-QUIZ COMPETITION ON CLINICAL ANATOMY

As the Covid-19 pandemic forced the entire country into a lockdown, the medical students had to deal with the frustrating concept of attending online classes from their homes. In order to motivate the students, the Department of Anatomy of Subharti Medical College, Meerut, organised the first national e-quiz competition on clinical anatomy for the First Phase MBBS students on Monday, 14th September, 2020.

The e-quiz was conducted via the Google Forms platform. The questions were based on the clinical anatomy related to gross anatomy, histology, neuroanatomy and embryology. The e-quiz comprised of 50 multiple choice questions arranged randomly in five sections of 10 questions each. The time allotted for the e-quiz was one hour.

The response towards the e-quiz was overwhelming as more than 1350 registrations from more than 150 medical colleges from India and abroad were received. The first, second and third rank holders were given prizes which were sponsored by the Rotary Club of Meerut Mahan. The top 50 rank holders were provided e-certificates for their performance.

The questions and their answers were made available on the journal's website after the completion of the e-quiz. The complete result list of all eligible contestants was also made available to assess their performance. The result of the top fifty rank holders is as follows.

Result of First National e-Quiz on Clinical Anatomy

Rank	FULL NAME	Name of the College	College State / U.T.
1.	MARATHE HARSH KIRAN	Seth GS Medical College, Mumbai	Maharashtra
2.	AMEY ABHIJIT AMBIKE	Seth GS Medical College, Mumbai	Maharashtra
3.	DEWANG AGARWAL	AIIMS Rishikesh	Uttarakhand
4.	NAYNESH MEENA	AIIMS Rishikesh	Uttarakhand
5.	ARYAN SINGLA	AIIMS Rishikesh	Uttarakhand
6.	KONIG SAMUEL	Government Vellore Medical College, Vellore	Tamil Nadu
7.	PRASHANT HARISH SARAF	Seth GS Medical College, Mumbai	Maharashtra
8.	DEBAYAN BANERJEE	Seth GS Medical College, Mumbai	Maharashtra
9.	VINAYAK MUKUND GODBOLE	Seth GS Medical College, Mumbai	Maharashtra
10.	ADITYA KUMAR SINGH	Patna Medical College and Hospital, Patna	Bihar
11.	SAYANTANI ROY	Malda Medical College & Hospital, Malda	West Bengal
12.	RAHUL DHILLON	King George's Medical University (KGMU), Lucknow	Uttar Pradesh
13.	KRISH KHERAJANI	Terna Medical College, Navi Mumbai	Maharashtra
14.	AISHWARYA GULANIKAR	B. J. Govt. Medical College, Pune	Maharashtra
15.	PARAS ARORA	Seth GS Medical College, Mumbai	Maharashtra
16.	MRUNMAYEE NERLIKAR	Kasturba Medical College, Manipal	Karnataka
17.	SATYAM MITTAL	National Institute of Medical Science & Research, Jaipur	Rajasthan
18.	ANEEKET MODAK	University College of Medical Sciences	Delhi
19.	ANJALI MISHRA	Government Medical College, Badaun	Uttar Pradesh
20.	PRATHAM AGRAWAL	Government Medical College, Nagpur	Maharashtra
21.	SHIKHAR GUPTA	King George's Medical University (KGMU), Lucknow	Uttar Pradesh
22.	KEERTANA ANAND	Kasturba Medical College, Manipal	Karnataka
23.	DEVANG CHHABRA	Lokmanya Tilak Municipal Medical College, Sion, Mumbai	Maharashtra
24.	NIKHIL KUMAR KENGUVA	Kasturba Medical College, Manipal	Karnataka
25.	SHRISTI KISHORE	JSS medical college, Mysore	Karnataka
26.	SHRADDHA GOYAL	Government Medical College, Badaun	Uttar Pradesh
27.	KALARAB MUKHERJEE	All India Institute of Medical Sciences, Kalyani, Nadia	West Bengal
28.	MAHAK BHANDARI	Lokmanya Tilak Municipal Medical College, Sion, Mumbai	Maharashtra
29.	NIKHIL FATTESING NAIK	Sri Bhausaheb Hire Government Medical College, Dhule	Maharashtra
30.	I VISHNU BHARADWAJ	Kasturba Medical College, Manipal	Karnataka
31.	PRAGYA MITRA	University College of Medical Sciences	Delhi
32.	HARSHITA RAMESH	Coimbatore Medical College, Coimbatore	Tamil Nadu
33.	CHARLIE GUPTA	Government Medical College, Faizabad	Uttar Pradesh

34.	ISHITA CHANDEL	Dr. Rajendar Prasad Government Medical College, Tanda	Himachal Pradesh
35.	YUMNA JAVED	Ganesh Shankar Vidyarthi Memorial Medical College, Kanpur	Uttar Pradesh
36.	VIDHI GUPTA	Maharashtra Institute of Medical Education & Research, Talegaon,Pune	Maharashtra
37.	DEBASMITA CHAKRABORTY	Burdwan Medical College, Burdwan	West Bengal
38.	VAIBHAV SINGH	King George's Medical University (KGMU), Lucknow	Uttar Pradesh
39.	FIDA FAISAL	Amrita Institute of Medical Sciences and Research Centre, Kochi	Kerala
40.	SHIKHAR S GUPTA	King George's Medical University (KGMU), Lucknow	Uttar Pradesh
41.	CHANDOLU PURNA NAGA SURYA VINAY	Great Eastern Medical School and Hospital, Srikakulam	Andhra Pradesh
42.	RAJEEV KUMAR	Dr. Baba Saheb Ambedkar Medical College, Rohini, Delhi	Delhi
43.	UMANG NATH CHAUBEY	King George's Medical University (KGMU), Lucknow	Uttar Pradesh
44.	NISHANTH R SUBASH	King George's Medical University (KGMU), Lucknow	Uttar Pradesh
45.	SRIRAM. D	Sri Muthukumaran Medical College,Chennai	Tamil Nadu
46.	MANASVI RAWAL	Lokmanya Tilak Municipal Medical College, Sion, Mumbai	Maharashtra
47.	SHIVANI SINGH	Amrita Institute of Medical Sciences and Research Centre, Kochi	Kerala
48.	DIVYANSHU SHUKLA	Ganesh Shankar Vidyarthi Memorial Medical College, Kanpur	Uttar Pradesh
49.	KEVIN EDWIN SAM	Teerthanker Mahaveer Medical College, Moradabad	Uttar Pradesh
50.	SRINIVASA IKSWAJA CHELLURI	Bharati Vidyapeeth Deemed University Medical College & Hospital, Sangli	Maharashtra

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