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Evaluation of Fertility Hormonal Profile on Women with Intramural Fibroid in Owerri

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Abstract
This study was carried out to evaluate levels of fertility hormonal profile in women with intramural fibroid in owerri. A total of seventy-five (75) subjects aged 18 – 50 years were recruited for the study and were divided into two groups subjects with intramural fibroid and control subjects. The serum follicle stimulating hormone, luteinizing hormone, Estradiol and Prolactin were assayed using ELISA Method. The data was analyzed using SPSS Version 21.0. The probability p=0.005 was statistically significant. The result obtained showed a significant increase (p=0.005) in follicle stimulating hormone (9.37±0.37miu/ml) in subjects with intramural fibroid (test) when compared with follicle stimulating hormone (6.72±0.48miu/ml) in control. There was a significant increase (p=0.005) in luteinizing hormone (6.19±0.27iu/ml) in test when compared with luteinizing hormone (5.36±0.21iu/ml) in control. There was a significant increase (p=0.005) in Estradiol (207.81±218.694pg/ml) in test when compared with Estradiol (88.14±16.79pg/ml) in control. There was a significant increase (p=0.005) in prolactin (73.34±9.68µg/l) in test when compared with Prolactin (14.67±10.96µg/l) in control. It was however confirmed that patients with intramural fibroid have increased serum levels of follicle stimulating hormone, luteinizing hormone, estradiol and prolactin. Therefore, it was concluded that intramural fibroid affects higher serum concentration of follicle stimulating hormone, leitinizing hormone, estradiol and prolactin due to this. It can contribute to the reduction of fertility or risk of miscarriage, complicated delivery in women with intramural fibroid.

Keywords: *fertility hormonal profile on women with intramural fibroid*

Introduction
Uterine fibroids (UFs), benign, monoclonal tumours of the female genital tract, originate from the myometrium.[1-3] Uterine leiomyomas (fibroids) represent the most common solid pelvic tumours and are associated with abnormal uterine Frankfurt bleeding and infertility. Myomas arise in 25–30% of women 4 during the reproductive years and constitute a frequent indication- for hysterectomy.[4-5]

Myomas are oestrogen-dependent, rarely seen before puberty and their progression stops after menopause.[6] Raised levels of oestrogens are believed to belong to the most important factors

inducing the formation and growth of UFs.[7-9] However, uterine fibroid growth was never observed under external administration of steroids only, indicating that pathophysiological pathways of uterine fibroid and other tumour formation is complex and unknown in many areas. Nowadays, a growing number of data consider progesterone to be a more important factor in initiating myometrial abnormal differentiation and growth than one of the major roles in the pathophysiology of uterine fibroid because they create a strong network of connections with progesterone.[7, 10] The essential role of oestrogens in the pathophysiology of uterine fibroid is confirmed by the fact that uterine fibroid rarely occurs before menarche and decrease after menopause. Moreover, a significant increase in uterine fibroid growth rates was observed in the hyperoestrogenic state. Similarly, a higher frequency of uterine fibroid was demonstrated in obese women with a high percentage of adipose tissue (strongly associated with hyperoestrogenism).[11]

Women who have high levels of both testosterone in midlife have an increased risk for developing incident uterine fibroids compared with women with low levels of the hormones, according to Jakimiuk *et al*..[12] Research suggests women undergoing the menopausal transition who have higher testosterone levels have an increased risk of developing fibroids, particularly if they also have higher estrogen levels.[11] The aim was to evaluate the levels of follicle stimulating hormone, luteinizing hormone and prolactinin women with intramural fibroid in Owerri.

Materials and Methods

Study Area

The study was carried out at Federal University Teaching Hospital Owerri Imo State.

Ethics, Advocacy and Pre-Survey Contacts

A letter of introduction was collected from the Head of Department of Medical Laboratory Science, Imo State University was collected (Appendix I) and submitted to the Head of Clinical Services and also Chairman Ethics Committee of Federal Medical Center, who subsequently granted an ethical approval for the study. A structured questionnaire was given to participants and those who gave consent to participate in the study were recruited.

Study Population

A total of seventy (75) subjects were recruited for the study, of which thirty (55) were subjects suffering from intramural fibroid while twenty (20) were control subjects.

Sample size determination

Sample size was determined in accordance to Araoye, (2004).

$$n=\frac{z^2pq}{d^2}$$

n=desired sample size

z= the standard normal deviate usually set at 1.96

p= the proportion of female with intramural fibroid in Owerri using a confidence interval set at 95% is 3.5%

q=1-p

d= degree of accuracy set at 0.05

n= 1.96^2x0.035x(1-0.035) = 3.84x0.035x0.965 =0.128= 51.2.

0.05^2 0.0025 0.0025

Minimum sample size was 51. individuals from the subject population.

Selection Criteria

A. Inclusion criteria

i. Female subjects confirmed of intramural fibroid, and had been attending clinic for not less than 3 months
ii. Subjects who were not diagnosed of other gynaecological pathologies like ovarian cancer.
iii. Subjects with intramural fibroid but not on any contraceptive pill.
iv. Subjects who were apparently healthy and served as control subjects
v. Subjects between the age of 18-50
vi. Subjects whose informed consent was obtained.

A. Exclusion criteria

i. Subjects below the age of 18 years and above 50 years.
ii. Subjects diagnosed of known gynaecological disorder.
iii. Female subjects on contraceptive pill.
iv. Subjects whose informed consent was not obtained.

Study Design

A cross-sectional study was conducted in the month of September 2023 and all eligible women who filled the questionnaire and gave a written informed consent for the study period were sampled. A total of 75 female subjects participated in the study. The study was grouped in two, group A representing (55) subjects suffering from intramural fibroid while twenty (20) are control subjects. After confirmed diagnosis of uterine fibroid, their blood samples were collected and used for the laboratory diagnosis of Oestrogen, FSH, LH and prolactin.

Sample Collection

Blood samples were collected aseptically by vein puncture, using a 5ml sterile disposable syringes and needles from petroleum attendants and non-petroleum attendants and were disposed into a labeled plain dry specimen container. The samples were centrifuged at 3,000rpm for 5 minutes to separate and to obtain the serum. The serum was extracted using a pipette and was introduced into another specimen container, and stored at -20^{oc} until required

Laboratory Procedures

All reagents used were commercially prepared and procured and the manufacturer's standard operating procedures were strictly followed.

A. Determination of Oestradiol using ELISA method according to as modified by Accubind, Lake Forest, USA CA 92630.

Procedure
100 µL of each Standard, Control and samples was disposed with new disposable tips into appropriate wells, 200 µL Enzyme Conjugate was dispensed into each well. It was mixed thoroughly for 10 seconds. It was incubated for 4 hours at room temperature; the contents were briskly shaked out of the wells. The wells were rinsed 3 times with diluted Wash Solution (400 µL per well). The wells were then Striked sharply on absorbent paper to remove residual droplets. 200 µL of Substrate Solution was added to each well, incubated for 30 minutes at room temperature and the reaction enzymatic was Stopped by adding 100 µL of Stop Solution to each well. The absorbance (OD) of each well was determined at 450 ± 10 nm with a microtitre plate reader. The wells were read within 10 minutes after adding the Stop Solution.

B. Determination of Luteinizing Hormone using ELISA Method according to as modified by Accubind, Lake Forest, USA CA 92630.

Procedure
Working solutions of the anti-hLH-HRP conjugate and wash buffer was prepared, the required number of microwells strips was removed. 25 µl of each calibrator, control and specimen sample was pipetted into correspondingly labelled wells in duplicate. 100 µl of assay buffer was pipetted into each well. The solution was Incubated on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature. The wells were washed 3 times with 300 µl of diluted wash buffer per well and the plate was tapped firmly against absorbent paper to ensure that it is dry. 100 µl of the conjugate working solution was pipette into each well, incubated on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature and the wells was washed again. 100 µl of TMB substrate was pipetted into each well at timed intervals and then incubated on a plate shaker for 15-20 minutes at room temperature (or until calibrator R attains dark blue color for desired OD.). 50 µl of stop solution was pipetted into each well at the same timed intervals as in step above. The plate was read on a microwell plate reader at 450 nm within 20 minutes after addition of the stop solution.

C. Determination of Follicle Stimulating Hormone Concentration using ELISA Method according to as modified by Accubind, Lake Forest, USA, CA 92630.

Test Procedure

All specimens and reagents were allowed to reach room temperature (25^0C) and mix thoroughly by gentle inversion before use. Standards, controls and samples should be assayed in duplicate. The micro titration strips were marked to be used and twenty-five microtiters of the standards, controls and samples were pipetted into the appropriate wells. One hundred micro-litres of the assay buffer E were added to each well using a semi-automatic dispenser. The wells were Incubated, shaked at a fast speed (500-700 rpm) on an orbital microplate shaker, at room temperature (~25^0C) for 2 hours. It was aspirated and washed each well 5 times with the wash solution using an automatic microplate washer. Blotted dry by inverting plate on absorbent material. The antibody-enzyme conjugate solution was prepared by diluting the antibody-enzyme conjugate concentrate in the assay buffer then, one hundred microliters of the antibody-enzyme conjugate solution was added to each well using a semi-automatic dispenser. The wells were incubated, shacked at a fast speed (500-700 rpm) on an orbital microplate shaker, at room temperature (~25 C) for 1 hour, aspirated and washed each well 5 times with the wash solution using an automatic microplate washer. Blot dry by inverting plate on absorbent material. One hundred microliters of the TMB chromogen solution were added to each well using a semi-automatic dispenser, the wells were incubated, shaked at a fast speed (500-700 rpm) on an orbital microplate shaker, at room temperature (~25C) for 10 minutes. Avoid exposure to direct sunlight. One hundred microliters of the stopping solution (0.2M sulfuric acid) was added to each well using a semi-automatic dispenser and the absorbance of the solution in the wells was read within 30 minutes, using a microplate reader set to 450 nm.

D. Determination of serum prolactin using ELISA method according to as modified by Accubind, Lake Forest, USA, CA 92630.

Procedure

Prior to assay, all reagents were allowed to stand at room temperature. All the reagents were gently mixed before use. The desired number of coated strips was placed into the holder, Pipette 25 µl of Prolactin standards, control and patient's sera was pipetted out, 100 µl of enzyme conjugate was added to all the wells and covered. The plate was incubated for 60 minutes at room temperature (25° C), liquid was removed from all wells, washed wells three times with 300 µl of 1X wash buffer. The plate was blotted on absorbance paper or paper towel and 100 µl of TMB substrate to all wells. It was incubated for 15 minutes at room temperature and 50 µl of stop solution was added to all wells. The plate was gently shake to mix the solution. Absorbance was read on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

Statistical Analysis

All values were expressed as mean ± standard deviation. The results were analyzed for statistical significance using the student T-test. P values <0.05, were considered statistically significant (Appendix IV). The statistical analysis was carried out using statistical packages for social sciences (SPSS) version 21.0

Results

Table 1: Mean ± standard deviation of fertility hormonal profile on women with intramural fibroid in the study population

Parameter	Intramural fibroid subjects n=50	Control subjects n=25	t-value	p-value (p=0.005)
Follicle Stimulating Hormone(mIU/ml)	9.37±0.37*	6.72±0.48	28.20	0.001
Luteinizing Hormone(IU/ml)	6.19±0.27*	5.36±0.21	14.78	0.001
Prolactin (µg/l)	73.34±9.68*	14.67±10.96	25.62	0.001
Estrodiol (pg/ml)	07.81±218.694*	88.14±16.79	3.03	0.003

The mean value of Follicle stimulating hormone, Luteinizing hormone, Prolactin and Estrodiol was significantly higher in Intramural Fibroid subject when compared to control.

Key:*=P=0.005 statistically significant

=sample size

Table 1 shows the mean ± standard deviation of fertility hormonal profile on women with intramural fibroid in the study population. The result showed that the mean value of follicle stimulating hormone is higher in intramural fibroid subjects (9.37±0.37pg/ml) which was statistically significant (p=0.001) when compared with the control subjects (6.72±0.48). The mean value of leuitnizing hormone was statistically significantly higher (p=0.001) in intramural fibroid subjects (6.19±0.27) when compared with the control subjects (5.36±0.21) The result showed that the mean value of prolactin is higher in intramural fibroid subjects (73.34±9.68) which was statistically significant (p=0.001) when compared with the control subjects (14.67±10.96). The mean value of Estrodiol was statistically significantly higher (p=0.003) in intramural fibroid subjects (207.81±218.694) when compared with control subjects 988.14±16.79).

Table 2: Correlation of Estrodiol with Follicule stimulating hormone, Luitinizing hormone and Prolactin in Intramural Fibroid Subjects

Variable	N	r	p-value
Follicule stimulating hormone	55	0.14	0.307
Luitinizing hormone	55	0.11	0.450
Prolactin	55	0.01	0.968

There was no significant relationship between Estradiol with follicle stimulating hormone, Letinizing hormone and prolactin in the study population. There was positive non-significant correlation between estradiol and follicle stimulating hormone in intramural fibroid (n=0.14, p=0.307) There was positive non-significant correlation between estradiol and follicle stimulating hormone in intramural fibroid (r=0.11, p=0.450) There was positive non-significant correlation between estradiol and follicle stimulating hormone in intramural fibroid (r=0.01, p=0.968)

Discussion

The result of the present study (table 1) showed a significant higher increase in the mean values of follicle stimulating hormone in intramural fibroid when compared with the mean value of the production of follicle stimulating hormone associated with intramural fibroid that can affect ovulation release that may lead to infertility. This present study also showed a significant increase in the luteinizing hormone when compared with the mean value of luteinizing hormone of the control indicating that this hormone can contribute to the growth of intramural fibroid, this is in agreement with the work done by Wallach *et al*[13] who showed that luteinizing hormone in premenopausal women stimulate intramural fibroid. Therefore, women with higher LH were more likely to have intramural fibroid. Their work also showed that intramural fibroid regrowth was seen in patient, luteinizing releasing hormone was used as a medical treatment procedure. The study also showed a significant increase in the mean value of prolactin in intramural fibroid subjects.

This present study also showed a significant increase in the estradiol when compared with the mean values of estradiol of the control indicating close relationship between the hormone and intramural fibroid.

Conclusion

The higher increase observed in this fertility hormonal profile can contribute to infertility, risk of miscarriage, complicated delivery. The findings have made significant contribution to the understanding of fertility hormone changes in women with intramural fibroid.

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