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RESEARCH ARTICLE

LEVELS OF TESTOSTERONE, FEMALE SEX HORMONES AND SOME TRACE ELEMENTS IN WOMEN WITH UTERINE FIBROIDS

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Abstract

Previous studies indicate that uterine fibroid is associated with estrogen and other female reproductive hormones and abnormal cell proliferation. This study is aimed at determining hormones of the pituitary/gonadal axis and some trace elements in women with uterine fibroids. The study was conducted at the Obstetrics and Gynaecology unit of the Federal Medical Centre, Owerri. A total of fifty (50) subjects were recruited for the study, their blood samples were collected and used for the laboratory diagnosis of testosterone, oestrogen, Follicle Stimulating Hormone (FSH), Leutinizing Hormone (LH) and trace elements. All reagents used were commercially prepared and procured and the manufacturer's standard operating procedures were strictly followed. All data generated from this study were subjected to statistical analysis, mean, Standard deviations, student's t-Test, and were determined using SPSS Statistical package software for windows version 21. Results shows that serum estrogen concentration (307.71 + 71.73 mIU/L) and Testosterone concentration (3.90+ 1.08 ng/ml) was significantly higher in uterine fibroid when compared with the serum concentrations of Oestrogen (173.67+ 33.98 mIU /L) and testosterone (1.26+ 0.39 ng/ml) in control subjects at p=0.002 and p=0.000 respectively. The mean value of serum FSH (4.45+ 0.72 IU/ml) and LH (3.15+ 0.84 IU/ml) was significantly lower in uterine fibroid when compared with the mean value of serum FSH (10.69+ 2.64 IU/ml) and LH (6.10+ 0.79 IU/ml) in control subjects at p=0.000 and p=0.000 respectively. The mean value of serum copper (5.99+ 0.62 µg/dl) and zinc (7.86+ 0.76µmol /L) was significantly lower in uterine fibroid when compared with the mean value of copper (8.56+ 1.19 µg/dl) and Zinc (9.25+ 0.83 µmol /L) in control subjects at p=0.000 and p=0.004 respectively. There is an alteration in the level of testosterone, estrogen, FSH, LH and trace element in women with uterine fibroid. In conclusion, increased circulating levels of testosterone with estradiol in women of child bearing age can be a higher risk factors in the development of uterine fibroid.

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Introduction:-

Uterine fibroid is the most common benign tumor of female reproductive organs with a clinical incidence of 20%-40%, and a detection rate of 77% for pathology (Stewart et al., 2016).Uterine fibroids (UFs), benign, monoclonal tumours of the female genital tract, originate from the myometrium (Stewart et al., 2016). 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 (Buttram and Reiter, 2011).These tumours are a major public health problem, due to the symptoms and costs they generate. The most common UF-dependent symptoms are abnormal, excessive uterine bleeding, pain, infertility, and obstetric pathologies (Stewart et al., 2017).The enormous healthcare budget burden related to UFs includes the costs of follow-up visits, diagnosis, treatment, and work absenteeism.

Numerous reports have been published on the risk factors for UF occurrence (Al-Hendy et al., 2017). Advanced age, increased body mass index (BMI), positive family history, and genetic predisposition are some of the most important risk factors for UF occurrence. According to available high-quality data, fibroid growth depends mostly on steroid hormones (Bulun, 2013).Myomas are oestrogen-dependent, rarely seen before puberty and their progression stops after menopause (Buttram and Reiter, 2011). Raised levels of oestrogens are believed to belong to the most important factors inducing the formation and growth of UFs (Borahayet al., 2017). 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 (Borahayet al., 2017). The essential role of oestrogens in the pathophysiology of uterine fibroid is confirmed by the fact that uterine fibroid rarely occur 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) (Stewart et al., 2017).

Oestrogens influence cellular physiological and pathological pathways through numerous different mechanisms. However, the most important action is binding to specific receptors (Jakimiuket al., 2007). Oestrogens interact with oestrogen receptor (ESR) α and ESRβ, which are members of the nuclear receptor family of intracellular receptors.Induction of oestrogen deprivation by chronic administration of luteinizing hormone-releasing hormone (LH-RH) agonists can be used for management of leiomyomas in pre-menopausal women (Healy et al., 2014).

However, cessation of therapy with LH- RH agonists and return to ovulatory menstruation are generally accompanied by rapid regrowth of fibroids. Moreover, pituitary desensitization, hypo-oestrogenism and shrinkage of uterine fibroids or reduction in uterine volume may be achieved in most patients only after 4–8 weeks surgical myomectomy. LH-RH and FSH-RH antagonists followed for up to 25 months and only in one case has the act more rapidly than the agonists, produce an immediate uterine volume increased after therapy(Jakimiuket al., 2007).

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 Jakimiuket al., (2007). 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(Stewart et al., 2017).

Mineral elements are crucial for many body functions which include transportation of oxygen, normalizing of the nervous systems, stimulation of growth and maintenance and repair of tissues and bones. Some of these elements are needed in small-amounts (few milligrams) per day, because when it is absorbed in excess, it is toxic to health and could cause damage to body system (Henry, 2011). Examples of mineral elements include; Zn, Fe, Cu, Mg, and Na. Some of these serve as cofactors for many enzymes (Cohn and Nay, 2010). A study by Akinlua and Ojo, (2013) stated that mineral elements; Na, Ca, K and Fe are implicated in fibroid cases. Hence diet and supplements containing these should be considered in managing fibroid patients.

Previous studies indicate that uterine fibroid is associated with estrogen and other female reproductive hormones and abnormal cell proliferation. More recent studies have implicated high testosterone level in the pathogenesis of uterine fibroid. However, the exact pathogenesis still remains unclear so far. There is paucity of information on the

level of trace element in women suffering from uterine fibroid hence this study is aimed at determining the level of hormones of the female sex hormone, testosterone and trace elements in women suffering from uterine fibroid.

Materials and Methods:-

Subjects:

A cross-sectional study was conducted in the month of September 2019 and all eligible women who filled the questionnaire and gave a written informed consent for the study period were sampled. A total of 50 female subjects participated in the study. The study was grouped in two, group A representing (30) subjects suffering from uterine fibroid while twenty (20) are healthy subjects (controls). After confirmed diagnosis of uterine fibroid, their blood samples were collected and used for the laboratory diagnosis of testosterone, oestrogen, FSH, LH and trace elements.

Selection Criteria

Inclusion criteria

1. Female subjects confirmed of Uterine fibroid, who have not gone for surgery.
2. Subjects who have not been diagnosed of other gynaecological pathologies like ovarian cancer.
3. Subjects with uterine fibroid but not on any contraceptive pill.
4. Subjects between the age of 18-50
5. Subjects whose informed consent was obtained.

Exclusion criteria

1. Subjects below the age of 18 years and above 50 years.
2. Subjects diagnosed of known gynaecological disorder.
3. Female subjects on contraceptive pill.
4. Subjects whose informed consent was not obtained.

Sample Collection and preparation

Blood samples were collected aseptically by vein puncture, using a 5ml sterile disposable syringes and needles from petroleum attendants and non-petroleum attendants and was disposed into a labelled plain dry specimen container. The samples were centrifuged at 3,000rpm for 5 minutes to separate and to obtain the serum. The serum were 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.

Determination of oestradiol using ELISA method (Accu-bind, OEST-6600)

Principle of the test

The Oestradiol sensitive ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with a polyclonal antibody directed towards an antigenic site on the Estradiol molecule. Endogenous Estradiol of a patient sample competes with an Estradiol horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is reverse proportional to the concentration of Estradiol in the sample. After addition of the substrate solution, the intensity of colour developed is reverse proportional to the concentration of Estradiol in the patient sample.

Calculation of results:

$$\text{Conc. of serum oestradiol} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of Std}$$

The standard curve was constructed as follows: The absorbance for the standards (vertical axis) versus the standard concentrations (horizontal axis) was plotted on a linear graph paper.

Determination of Testosterone Concentration using ELISA Method (ACCU- bind ELISA assay kit catalog number: 3725-300) (ACCU-bind, 2016)

Principle of the test

The Testosterone ELISA is based on the principle of competitive binding between Testosterone in the test specimen and testosterone-horseradish peroxidase (HRP) conjugate for a constant amount of rabbit anti-Testosterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with testosterone standards, controls, patient samples, testosterone-HRP conjugate reagent and rabbit anti-testosterone reagent for 90 minutes. During the incubation, a fixed amount of HRP-labeled testosterone competes with the testosterone in the standard, sample, or quality control serum for a fixed number of binding sites of the specific testosterone antibody. Thus, the amount of testosterone-HRP immunologically bound to the well progressively decreases as the concentration of Testosterone in the specimen increases. Unbound testosterone-peroxidase conjugate is then removed and the wells washed, followed by addition of TMB Reagent resulting in the development of blue color. The color development is stopped and the absorbance is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled testosterone in the sample. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The testosterone concentration of the specimens and controls run concurrently with the standards can be calculated from the standard curve (Nwanjo, 2008).

Calculation of results

Conc. of serum testosterone= Absorbance of test X Concentration of Std
Absorbance of standard

The standard curve was constructed as follows: The absorbance for the standards (vertical axis) versus the standard concentrations (horizontal axis) was plotted on a linear graph paper.

Determination of Luteinizing Hormone using ELISA Method (ACCU- bind (ELISA assay kit catalog number: 625-300) (ACCU-bind, 2016)

Principle of the test

The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for LH is immobilized onto the microwell plate and another monoclonal antibody specific for a different region of LH is conjugated to horse radish peroxidase (HRP). LH from the sample and standards are allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of LH in the sample. A set of standards is used to plot a standard curve from which the amount of LH in patient samples and controls can be directly read (Accu-bind ELISA kit).

Calculation of results:

Conc. of serum LH = Absorbance of test X Concentration of Std
Absorbance of standard

The standard curve was constructed as follows: The absorbance for the standards (vertical axis) versus the standard concentrations (horizontal axis) was plotted on a linear graph paper.

Determination of Follicle Stimulating Hormone Concentration using ELISA Method (ACCU- bind ELISA assay kit catalog number: 425-300) (ACCU-bind, 2016)

Principle of the test

A known antibody is adsorbed on a solid phase, to which the test sample which may contain the antigen is added. During incubation, the antibody captures or binds the antigen to the solid phase. After washing enzyme labelled specific antibody is added. At this stage, the antigen is sandwiched between two antibody molecule, one on the solid phase and the other with the enzyme label.

On addition of substates, a colour change indicates that the enzyme labelled antibody is present on the solid phase. This is due to the present of antigen in the test sample. The colour change was measured using a spectrophotometer at 450nm wavelength. The intensity of the colour is directly proportional to the concentration of the antigen present.

The enzyme activities were stopped after a period by changing the pH with acid

Calculation of results:

Conc. of serum FSH = Absorbance of test X Concentration of Std
Absorbance of standard

The standard curve was constructed as follows: The absorbance for the standards (vertical axis) versus the standard concentrations (horizontal axis) was plotted on a linear graph paper.

Determination Of Copper and Zinc Concentration using (AAS Method)

Copper and zinc level was determined in sample using the Atomic Absorption spectrophotometers (AAS) assay.

Principle of the test

The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on Beer-Lambert Law. In short, the electrons of the atoms in the atomizer can be promoted to higher orbitals (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given wavelength). This amount of energy, i.e., wavelength, is specific to a particular electron transition in a particular element. In general, each wavelength corresponds to only one element, and the width of an absorption line is only of the order of a few picometers (pm), which gives the technique its elemental selectivity. The radiation flux without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using Beer-Lambert Law. Upon introduction of the metal solution into the instrument, the solution is vapourised by the flame or a furnace, and the trace metal to be detected is dissociated from its chemical bonds into its elemental form. A hollow cathode or electrode less discharge lamp provides characteristic radiation energy for the metal. The wavelength of this emitted radiation must match the absorption wavelength of the metal to be determined. The amount of energy absorbed by the metal atoms is related to their concentration. Since each metal absorbs light at a characteristic wavelength, analysis for each metal requires a different light source, and only one element can be determined at a time (Cheesbrough, 2007).

Statistical Analysis

All data generated from this study were subjected to statistical analysis, mean, Standard deviations, student's t-Test, and were determined using SPSS Statistical package software for windows. Results were expressed as Mean + SD. The 5% ($P<0.05$) level was considered significant.

Results:-

Table 1:- Serum Estrogen, Follicle stimulating hormone, Luteinizing hormone and Testosterone of Uterine fibroid patients versus Controls (Apparently healthy Women).

Parameters	Test	Control	t-value	p-value
Estrogen (mIU /L)	307.71+ 71.73	173.67+ 33.98	4.97	0.002
FSH (mIU/mL)	4.45+ 0.72	10.69+ 2.64	-6.04	0.000
LH (mIU/mL)	3.15+ 0.84	6.10+ 0.79	-7.23	0.000
Testosterone (ng/ml)	3.90+ 1.08	1.26+ 0.39	6.79	0.000

Key:

FSH: Follicle Stimulating Hormone
LH: Luteinizing Hormone

Table 1 shows that serum estrogen concentration (307.71 + 71.73 mIU/L) and Testosterone concentration (3.90+ 1.08 ng/ml) was significantly higher in uterine fibroid when compared with the serum concentrations of Oestrogen (173.67+ 33.98 mIU /L) and testosterone (1.26+ 0.39 ng/ml) in control subjects at p=0.002 and p=0.000 respectively. The mean value of serum FSH (4.45+ 0.72 IU/ml) and LH (3.15+ 0.84 IU/ml) was significantly lower

in uterine fibroid when compared with the mean value of serum FSH (10.69+ 2.64 IU/ml) and LH (6.10+ 0.79 IU/ml) in control subjects at p=0.000 and p=0.000 respectively .

Table 2:- Serum levels of Copper and Zinc of Uterine fibroid patients versus Controls (Apparently healthy Women).

Parameters	Test	Control	t-value	p-value
Copper (µg/dl)	5.99+ 0.62	8.56+ 1.19	-5.16	0.000
Zinc (µmol /L)	7.86+ 0.76	9.25+ 0.83	-3.35	0.004

The mean value of serum copper (5.99+ 0.62 µg/dl) and zinc (7.86+ 0.76µmol /L) was significantly lower in uterine fibroid when compared with the mean value of copper (8.56+ 1.19 µg/dl) and Zinc (9.25+ 0.83 µmol /L) in control subjects at p=0.000 and p=0.004 respectively

Discussion:-

Although gonadotropins, adipokines, and ovarian peptides may be have some influence on fibroid onset and growth. Studies show a relationship between androgen and estrogen in the development of uterine fibroid.

The present study revealed that serum estrogen concentration was significantly higher ($p<0.05$) in uterine fibroid when compared with the control subjects. Studies by Gao et al., (2015) stated an assumption that estrogen is the main feeder of uterine leiomyomas. Otubu et al. found a significantly higher concentration of 17β-oestradiol in fibroids than normal myometrium, especially in the proliferative phase. The authors speculated that the higher levels of oestradiol in the fibroids could be related to lower levels of the enzyme 17β-hydroxysteroid dehydrogenase. Estrogen receptors are abundantly expressed in uterine leiomyomas, which ensures considerable responsiveness to the circulating estrogen. Whether the tumors are richer in estrogen receptors than the surrounding myometrium is still debatable, with some studies showing such a difference (Moraveket al., 2015). The result of this study is similar to the study conducted by Carret al., (2013)

The mean value of serum FSH and LH was significantly lower ($p<0.05$) in uterine fibroid when compared with the control subjects. Follicle stimulating hormone and luteinizing hormones are gonadotrophin hormones which stimulates and regulate the release of oestrogen, the present study reported an increase in oestrogen level in women with uterine fibroid, the increase in oestrogen causes a drop in the level of FSH and LH by negative feedback mechanism. The result of this study is similar to the study carried by (Stewart, 2011) in their study they found out a low level of FSH and LH in uterine fibroid subject, this is consistent with the finding of our result.

The present study reveals that, the mean value of serum Testosterone was significantly higher ($p<0.05$) in uterine fibroid when compared with the control subjects. The mechanism by which this happens is unknown, studies by Lee et al., (2010) reported that Women with high bioavailable testosterone had increased risk of incident fibroids compared to women with low testosterone. From a longitudinal study conducted by Lee et al., (2010), higher levels of bioavailable testosterone is related to an increased risk of incident fibroids in the ensuing years in women who never previously reported them, but is related to a decreased risk of recurrent fibroids in women who previously reported them. Lee et al., (2010) also suggested that testosterone and estrogen may act on receptors in undetected fibroids.

The present study reveals that the mean value of serum copper and zinc was significantly lower ($p<0.05$) in uterine fibroid when compared with the control subjects. Zinc plays a role through structural stabilization of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and ribosome. It has a protective effect against free-radical injury (Wu et al., 2014). The mechanism behind the low level of zinc and copper in uterine fibroid patients is unknown.

Copper plays a role in the production of haemoglobin, myelin, collagen and melanin as an essential nutrient and studies have shown that normal immune function requires adequate Cu intake (Zuoet al., 2016). The low level of serum zinc level might be as a result of it role in uterine growth, the over utilization of serum copper level leads to the low level of copper in patients with uterine fibroid. This study is similar to the research carriedd out Wu et al., (2014).

Conclusion:-

There is an alteration in the level of testosterone, estrogen, FSH, LH and trace element in women with uterine fibroid. From the result of this study, increased circulating levels of testosterone with estradiol in women of child bearing age can be a higherrisk factors in the development of uterine fibroid.

Recommendation:-

Further studies concerning the molecular mechanisms involved in the pathogenic process of uterine fibroid are required. A longitudinal study with a larger sample size is recommended in this study.

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