Original Research Article

Topic: Correlational analysis of Interleukin 6, Adiponectin and Lipid indices in women with Uterine Fibroids.

ABSTRACT

Background: Uterine fibroids are the most common benign, monoclonal tumours affecting women of reproductive age.

Aim: This study investigated the association between interleukin-6 (IL-6), adiponectin (ADP) and lipid indices in women with uterine fibroids.

S**ubjects and methods:** Sixty (60) participants were recruited for this study. They comprised thirty (30) subjects who had ultrasonographic evidence of uterine fibroids and thirty (30) subjects who had no ultrasonographic evidence of uterine fibroids. Subjects were recruited from the Gynaecology out-patient clinic of LAUTECH Teaching Hospital Ogbomoso, Nigeria. Anthropometric measurements were performed using standard method. About 5mL of venous blood was collected from each study participant and was dispensed into a plain bottle. Serum was obtained after clotting and centrifugation and was aliquoted into a small vial which was stored at -20^{o}C until time of analysis. Interleukin-6 (IL-6), adiponectin (ADP), total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL) were determined using enzyme linked immunosorbent assay (ELISA) and colorimetric method as appropriate. Low density lipoprotein (LDL) was estimated using Friedwald's equation. Data analysis was done using Student's t-test for comparison of variables and Pearson's correlation was used to determine the relationship between variables. *P*–value less than 0.05 was considered significant.

Results: Women with uterine fibroids had significantly elevated body mass index (BMI), waist circumference (WC) and waist-hip ratio (WHR) when compared with the controls ($P<0.05$). The mean values of TC, TG, LDL and IL-6 were significantly elevated in women with uterine fibroids when compared with the control subjects ($P<0.05$). Women with uterine fibroids had significantly reduced levels of ADP when compared with controls ($P<0.05$) and there was no significant difference in the levels of HDL when both case and control subjects were compared ($P>0.05$). Interleukin-6 had a significant positive correlation with BMI, WC, WHR, TC, TG and LDL. Interleukin-6 also had a significant negative correlation with HDL and ADP.

Conclusion: This study demonstrated a significant direct relationship between IL-6 and dyslipidemia in women with uterine fibroids. Also, we observed a noteworthy inverse relationship between IL-6 and

adiponectin in women with uterine leiomyomas, thus emphasizing the low grade chronic inflammatory state, associated with uterine leiomyomas.

Keywords: Uterine leiomyoma, interleukin-6, adiponectin, adipocytes, lipid profile.

1. INTRODUCTION

Uterine fibroids (leiomyomas) are benign, monoclonal tumors originating from the myometrium and the most common pelvic tumors of the female reproductive system (1). The symptoms of uterine fibroids include menstrual disturbances such as menorrhagia, dysmenorrhea, intermenstrual bleeding, dyspareunia and noncyclic pelvic pain (2) and pressure symptoms such as a sensation of bloatedness, increased urinary frequency, and bowel disturbances (3). It has also been associated with impaired reproductive outcomes, leading to subfertility, abortion, preterm labor and delivery, and cesarean delivery (3, 4). The estimated prevalence of uterine fibroids in Southwest Nigeria is 7.0% (5).

The occurrence of fibroids has been linked with the hormones: estrogens, and progesterone. These hormones are also responsible for fibroid growth. Some studies have reported the up-regulation of progesterone receptor-A (PR-A) and PR-B, oestrogen receptor-alpha (ER-α) and -beta (ER-β) as well as messenger RNA (mRNA) and protein expression in myoma tissue compared with normal myometrium (6, 7). Leiomyoma growth is influenced by progesterone interaction with some growth factors; it upregulates the endothelial growth factor (8) and transforming growth factor-beta 3 (bimodal action) expression (9). On one hand, progesterone seems to down-regulate interleukin growth factor-1 expression through PR-B while PRA appears to inhibit this function (8). Other factors influencing the development of myomas include: age, race, obesity, lifestyle such as smoking, alcohol and other drugs which can affect the uterine wall (10). It is therefore hypothesized that oestrogens exert growth stimulatory effects on leiomyomas intermediated by cytokines, growth factors, or apoptotic factors (11). The immune system plays an important role in the aetiology of leiomyomas. This includes the chronic inflammation associated with cytokine release by the immune system as well as the undifferentiated cells (5, 12, 13).

In obesity, adipocytes undergo molecular and cellular alterations which includes hypertrophy; with resultant effect on systemic metabolism, thus leading to insulin resistance, metabolic disorders and inflammatory responses due to the release of pro-inflammatory factors from the adipocytes (14). Also, adipocytes secrete adiponectin and previous studies have demonstrated that adiponectin has insulin-sensitizing and anti-inflammatory effects (15, 16).

Since obesity has been implicated as a predisposing factor for leiomyomas, there is likelihood of dyslipidaemia. Also, studies have reported a link between tumor necrosis factor-alpha (TNF-α), a pro

inflammatory cytokine, and the growth of uterine fibroid (17, 18). This study is therefore focused on determining adiponectin, Interleukin-6 and lipid indices in order to obtain valuable information on the correlation between them in women with uterine fibroids.

2. MATERIAL AND METHODS

2.1 Subjects

A total of sixty (60) participants were recruited for this case-control study. They comprised thirty (30) subjects who had ultrasonographic evidence of uterine fibroids and thirty (30) subjects who had no ultrasonographic evidence of uterine fibroids. Subjects were recruited from the Gynaecology out-patient clinic of LAUTECH Teaching Hospital Ogbomoso, Nigeria. Subjects were infertile women referred for ultrasound examination and based upon this examination, they were stratified into women with fibroid (case) and women without fibroid (control). All the subjects had normal menstrual cycle and they were not on any hormonal medication. All the subjects consented to participate in the study through writing.

A short structured questionnaire was administered to each study participant to obtain information on smoking habits, medications and established diseases. Persons diagnosed with dyslipidemia and other metabolic conditions, record of alcoholism, smoking, usage of medications that affect lipid status, pregnant women and those who refuse to give consent were excluded from this study.

2.2 Anthropometric measurement

Body weight, height, body mass index (BMI), waist circumference (WC) and waist to hip ratio (WHR) were obtained from the participants by standard methods as described by Charles-Davies *et al*. (19).

2.3 Sample Collection and Biochemical Analyses

Blood sample (5mL) was collected and dispensed into a plain bottle. Serum was obtained after clotting and centrifugation and was aliquoted into a small vial which was stored at -20^{o}C until time of analysis. Serum Total Cholesterol (TC) and Triglyceride (TG) were determined using standard enzymatic method while high density lipoprotein-cholesterol (HDL-C) was determined by a two-step procedure as previously described by Adediji *et al*. (20). LDL cholesterol was estimated using Friedewald's equation; [Total cholesterol – HDL cholesterol – (Triglyceride/ 5) mg/dL].

Serum Adiponectin and IL-6 were determined using ELISA kits manufactured by Elabscience Biotechnology Co. Ltd (Wuhan, China) with the procedure as previously carried out by Ashraf *et al*. (21).

2.3.1 Principle of ELISA

In this assay, the antigen – adiponectin or IL-6 present in each sample reacts with the corresponding antibody adsorbed to the surface of solid-phase polystyrene microtitre wells. On removal of unbound

proteins by washing, the antibodies conjugated with horseradish peroxidase (HRP) form complexes with the previously bound antigen following the addition of a chromogenic substrate, 3, 3, 5, 5-tetramethylbenzidine (TMB). The absorbance at 450 nm is a measure of the concentration of the 'antigen' in the test sample.

2.3.2 ELISA Procedure

A gradient of standard concentrations were prepared from the concentrated standard through serial dilution to cover the expected assay range. One hundred microliters of the standards and sera were pipetted into microwells already coated with specific antibodies and incubated at 37°C for 90 min. Following incubation, the wells were aspirated of their contents without washing and 100µl of biotinylated detection antibody was added to each well and incubated for 60 minutes at 37°C, after which each well was completely filled with appropriate wash solution. The plate was washed three times.

One hundred microliters of appropriately diluted enzyme–antibody conjugate was pipetted into each well and the plate was incubated at 37°C for 30 minutes. After incubation, another process of washing was performed as described above and 90µl of TMB substrate solution was added to each well. This was followed by incubation for 15 minutes at 37°C after which 50µl of stop solution was added to each well. The absorbance (at 450 nm) was determined using a microplate reader.

2.4 Statistical analysis

Data analysis was done using SPSS version 20 (IBM, Armonk, NY, USA). All values were expressed as mean±standard deviation for test and control groups. Comparison of variables was done using Student's t-test and Pearson's correlation was used to determine the relationship between variables. *P* less than 0.05 was considered to be statistically significant.

3. RESULTS

The anthropometric and biochemical parameters of the study participants are shown in table 1. The mean values of BMI, WC, WHR, TC, TG, LDL and IL-6 were elevated in women with uterine fibroids when compared with the control subjects. The mean values of ADP were significantly reduced in women with uterine fibroids when compared with the controls and there was statistical significant difference in the levels of HDL when both test and control subjects were compared.

Table 2 shows the correlation IL-6, anthropometric and other biochemical parameters in women with uterine fibroids. IL-6 had a significant positive correlation with BMI (R=0.362, *P*=0.03), WC (R=0.456, *P*=0.02), WHR (R=0.374, *P*=0.04), TC (R=0.735, *P*=0.00), TG (R=0.429, *P*=0.01), LDL (R=0.606, *P*=0.04). Also, IL-6 had a significant negative correlation with HDL (R= -0.590, *P*=0.03) and ADP (R= -0.527, *P*=0.02) in women with uterine fibroids.

Table 1: Anthropometric and biochemical parameters in study participants.

Parameters	Subjects (n=30)	Control (n=30)	*P*-value
BMI (kgm-^{2})	28.6 ±2.76	24.5 ±4.68	0.04*
WC (cm)	86.3 ±11.3	77.5 ±8.7	0.02*
WHR	1.15±0.08	1.04±0.13	0.00*
TC (mg/dL)	193.1±24.2	146.3±38.1	0.00*
TG (mg/dL)	180.6±29.9	131.6±45.6	0.00*
HDL (mg/dL)	42.5±5.43	43.3±3.38	0.56
LDL (mg/dL)	114.5±19.5	76.5±31.0	0.00*
IL-6 (pg/mL)	352.6±39.7	233.5±43.6	0.00*
ADP (µg/L)	121.9±33.3	189.2±81.8	0.00*

**Statistically significant at P<0.05. Results are expressed as mean±standard deviation. BMI-Body mass index; WC- Waist circumference; WHR- Waist to hip ratio; TC- Total cholesterol; TG- Triglycerides; HDL- High density lipoprotein; LDL- Low density lipoprotein; IL-6- Interleukin-6; ADP- Adiponectin.*

Table 2: Correlation between IL-6, anthropometric and other biochemical parameters in women with uterine fibroids

Parameters	R	*P*– value
BMI†	0.362	0.03*
WC†	0.456	0.02*
WHR†	0.374	0.04*
TC†	0.735	0.00*
TG†	0.429	0.01*
HDL†	-0.590	0.03*
LDL†	0.606	0.04*
ADP†	-0.527	0.02*

**Statistically significant at P<0.05. BMI†= Correlation between IL-6 and BMI; WC†= Correlation between IL-6 and WC; WHR†= Correlation between IL-6 and WHR; TC†= Correlation between IL-6 and TC; TG†= Correlation between IL-6 and TG; HDL†= Correlation between IL-6 and HDL; LDL†= Correlation between IL-6 and LDL; ADP†= Correlation between IL-6 and ADP.*

4. DISCUSSION

Uterine fibroids continue to be the most common gynecological benign tumours in women of reproductive age and there are multiple risk factors which have been implicated in the development of uterine leiomyoma.

In this study, body size and central obesity were measured by BMI, WC and WHR and we observed increased BMI, WC and WHR in women with uterine fibroids when compared with controls. These findings are consistent with previous studies that reported a significant positive association between central obesity and incidence of uterine fibroids (22-24). This observation could be attributed to increased adiposity which accentuates the aromatization of androgens to estrogens and the consequent hyperestrogenic state is responsible for the growth of uterine fibroids in obese women (25-27).

Furthermore, the progressive adipocyte hypertrophy accentuates low grade chronic inflammation which is thought to play a major role in the progression of uterine fibroids. Adipocytes secrete proinflammatory cytokines like IL-6 and they also secrete anti-inflammatory mediators like adiponectin. In our study, we observed a significant increase in the levels of IL-6 and a significant reduction in the levels of adiponectin, when women with uterine fibroids were compared with the controls. This observation is further supported by our observed significant inverse relationship between IL-6 and adiponectin in women with uterine fibroids. The observed elevated IL-6 could be attributed to excessive secretion of IL-6 by peritoneal macrophages which are abundantly present in enlarged adipocytes and are actively involved in their activities (28-30). IL-6 and other proinflammatory factors produced due to excessive fat accumulation have a direct effect on the myometrium and this consequently enhances the growth of uterine leiomyomas (4, 30).

Moreover, the observed low serum adiponectin could also be attributed to increased adiposity which promotes excessive secretion of IL-6 and this in turn, stymies the production of adiponectin in adipocytes (21, 31). Previous studies have reported that low adiponectin levels has a direct relationship with increased insulin levels and insulin is a potent regulator of sex hormone binding globulin (SHBG) (31, 32). Thus, increased insulin level attenuates hepatic production of SHBG, leading to an increase in the circulating levels of androgens which further enhances the growth of uterine leiomyomas in obese women (24, 33).

In this study, we also observed significant increase in the levels of TC, TG and LDL when the case subjects were compared with controls however, there was no statistical significant difference in the levels of HDL-C. We also observed a significant positive correlation between IL-6, TG, TC and LDL while there was significant inverse relationship between IL-6 and HDL-C in women with uterine fibroids. These observations are consistent with previous studies (23, 24, 27) and could be attributed to the influence of

estrogen on TG accumulation and the ability of IL-6 to accentuate lipid abnormalities through its pronounced inhibitory effect on the activities of adipocyte lipoprotein lipase. (34-36).

5. CONCLUSION

This study revealed a significant direct relationship between IL-6 and dyslipidemia in women with uterine fibroids. Also, we observed a noteworthy inverse relationship between IL-6 and adiponectin in women with uterine leiomyomas, thus emphasizing the low grade chronic inflammatory state associated with uterine leiomyomas.

The major limitation of the study was sample size, therefore a cross sectional study on a larger population is desirable in confirming our findings.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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