

ARTICLE

Clinicopathological Patterns and Biochemical Markers in Serum of Uterine Leiomyoma Patients

Sundas Akram¹, Amaila Akmal¹, Shaaf Ahmad², Husna Ahmad¹, & Nabila Roohi¹¹Institute of Zoology, University of the Punjab, Quaid-i-Azam, Campus, Lahore, 54590, Pakistan²Mayo Hospital, King Edward Medical University, Hospital Road, Lahore, 54000, Pakistan

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*nabilruhi@gmail.com



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Competing interests

The authors have declared that no competing interests exist.

Abstract

Background: Uterine fibroids (leiomyomas) are exceedingly common reproductive neoplasms with foremost public health impact. A cross-sectional study was performed to systematically investigate the marker enzymes, clinicopathological correlations, and electrolyte profile in myoma

Method: This study enrolled 44 control and 46 leiomyoma subjects, aged 21- 50 years. Anthropometric parameters, detailed history of disease and clinicopathological outcomes were documented via a standardized questionnaire followed by uterine ultrasound investigation. Venous blood samples were taken for the measurement of marker enzymes and serum electrolyte concentration with commercially accessible kits

Results: In the age group between 30-40 years high incidence of myoma (43.5%) was found. Majority of fibroids were observed single (52%) and Intramural uterine fibroids were found more common type (61%) of leiomyomas. Menorrhagia was frequent clinical demonstration with 63% leiomyoma cases. In 26% myoma women positive family history of leiomyomas was also observed. A significant increase in diastolic blood pressure (DBP) and body mass index (BMI) while in parity a significant decrease was recorded in leiomyoma patients in comparison with controls. Serum electrolytes investigation revealed a substantial increase in the calcium (Ca^{2+}) as well as chloride (Cl^-) concentration and significant drop in potassium (K^+) concentration in myoma subjects when compared to the controls. While for serum sodium (Na^+) concentration, a non-significant difference was documented between comparable groups. Analysis of marker enzymes manifested a significant increase in the serum concentration of aspartate transaminase (AST), alanine transaminase (ALT) and acid phosphatase (ACP) in fibroid patients in comparison to controls whereas non-significant variations were recorded for serum alkaline phosphatase (ALP) concentration.

Conclusion: A reduced serum K^+ concentrations and raised Ca^{2+} , Cl^- and Na^+ levels in the leiomyoma patients illustrate increased estrogen concentration, that may be responsible for fibroid growth and serum concentration of AST, ACP and ALP are sustainable diagnostic markers of uterine fibroids.

Key words: ALP, ALT, AST, Calcium, Potassium, Sodium, Uterine leiomyoma

Introduction

Uterine fibroids (leiomyomas), the most common benign muscular tumors arising from the uterine wall, are characterized by low mitotic index and high production and accumulation of irregularly arranged extracellular matrix (Yafang & Nadarajah, 2022; Halder et al., 2011). Globally, these fibroids are one of the leading gynecological problems. These fibroids affect about 77% of women in reproductive period (Ciarmela et al., 2011). Pathogenesis of these fibroids is multifactorial, and the exact etiology associated with its genesis and growth is still unclear. Nonetheless, epidemiologic evidence suggests that interactions between numerous cytokines, genes, growth factors and hormones like progesterone, estrogen and corticosterone are the main factors responsible for fibroid growth (Shen et al., 2016). Uterine leiomyomas arise as a solitary benign tumor or in groups and can be submucosal (uncommon), intramural (develop in muscular uterine wall) and subserosal. Multiple clinical risk factors including parity, premeno-

pausal status, hypertension, increasing age, early age at menarche, obesity and ethnicity have been reported to be associated with its pathogenesis (Boyles et al., 2021; Noel et al., 2019; Flake et al., 2003).

Dietary minerals and lifestyle factors have a significant effect on the expansion and development of the fibroids (Szydłowska et al., 2022; Okolo, 2008). Electrolytes are chemical fundamentals that are vital for the growth and development of living organisms (Skinner, 2005; Newman & Benfield, 2002). In fibroids patients' variation in electrolytes such as calcium (Ca^{2+}), sodium (Na^+), potassium (K^+), phosphorus (P), magnesium (Mg^{2+}) and chloride (Cl^-) occur at cellular and molecular level. Estrogen and progesterone levels increases in fibroid patients. These hormones along with some other growth factors are actually responsible for electrolytes variations especially (Na^+ , K^+ and Cl^-) in leiomyoma patients. In addition, calcium excretion through kidney decreases because of parathyroid hormone related protein (PTHrP) leading to hypercalcemia (Ke et al., 2014; Akinlua & Ojo, 2013). Pelvic pain, premature labor eventually leading to infertility, abdominal distention are common complications linked with fibroids (Giuliani, 2020). Among fibroid patients' menorrhagia also prevails because of elevated blood level of chloride, sodium (Hapangama & Bulmer, 2016). Enzymatic changes inside uterine tissues also responsible for development of fibroids. For identification and management of a disease, marker enzymes are typically used. Enzymes such as aspartate aminotransferase (AST), acid phosphatase (ACP), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), can be used as a tumor marker. Normal cells produce these marker enzymes in small concentration than tumor cells (Yoo et al., 2020; Ojo & Oyeyemi, 2013).

The aim of this work was to study clinicopathological patterns and to evaluate the association of marker enzymes (ACP, ALT, ALP, and AST) and serum electrolytes (Na^+ , Ca^{2+} , K^+ , Cl^-) with uterine fibroids growth and development.

Materials and Methods

A cross-sectional study was conducted on 90 participants. The objectives and benefits of the study were explained to each of the participant before obtaining any information or blood sample and a written informed consent was signed by them prior to their recruitment in the study. Premenopausal women belonging to the same age group (20-52 years) as well as socioeconomic status were considered inclusion criteria for this study. Premenopausal status was defined as, occurrence of at least one menstrual cycle in the previous 12 months.

All of the participants were grouped into Control and Leiomyoma group. Forty-six premenopausal women with symptomatic uterine leiomyoma confirmed by ultrasound diagnosis and clear adnexa were recruited in the leiomyoma group. Women were included consecutively in leiomyoma group if they were diagnosed with at least one fibroid in uterus. Forty-four healthy reproductive age female volunteers with leiomyoma free uterine morphology, non-lactating, non-pregnant and without any gynecologic symptoms and selected as reference population were included in the control group.

A standardized questionnaire was designed which included information about the age, height and weight, parity, and history of diseases of the participants. Women who were younger than

18 years old, pregnant, postmenopausal with histopathological evidence of endometriosis, adenomyosis, pelvic inflammatory disease, ovarian or cervical malignancy or any other adnexal pathology were excluded. Women with hepatic, respiratory, renal, cardiac, and endocrine disorders as well as current genital infection were also excluded from the study. Leiomyoma patient with previous history of myomectomy or hysterectomy for the surgical intervention of uterine leiomyoma were also excluded. Medical records of fibroid patients were reviewed retrospectively and clinicopathological information covering number of leiomyomas, type of leiomyoma and presence/absence of menorrhagia, was abstracted from patient's medical records. Biological feature of anemia such as hemoglobin level was also recorded for each patient and Hb level was categorized according to the WHO prescribed classification as mild anemic (10.0-10.9), moderate anemic (8.1-9.9) and severe anemic (<8.00).

Body weight (kg) and height (m) were measured for the calculation of body mass index (kg/m^2). Body weight was measured using digital weighing machine. During weighing, subjects were wearing light clothing. Actual body weight of the subjects was calculated by subtracting average weight of clothing. Body height was recorded by using metric scale, with the participants standing in upright position without shoes. Body weight was compared and interpreted with reference to height (m) in order to calculate body mass index (kg/m^2).

After an overnight fasting, 5ml of venous blood, from all of the participants, was collected by using peripheral venous catheter. Blood samples were transferred to the for-serum collection vacutainer and allowed to clot for 30 min. After clotting, blood samples were centrifuged at 3000rpm for 10 min. Sera supernatants were separated, aliquoted and stored without the loss of activity at -81°C , until assayed. Serum electrolytes and marker enzymes were analyzed with the help of commercially available diagnostic kits, using clinical chemistry analyzer.

Statistical analysis

Graph pad prism (version 6.05) was used for the statistical analysis of parameters of comparable groups and unpaired Student t-test was employed for the determination of level of significance. The results were presented as Mean \pm SEM at 95% confidence interval (95% CI) and a p-value < 0.05 was considered statistically significant. Data obtained from questionnaire was entered, screened, and analyzed by using statistical software (SPSS, version 21) and descriptive analysis was performed using frequencies and percentages.

Results

The clinicopathological patterns of patients with uterine leiomyomas (n=46) are summarized in Table 1.

The mean weight of the leiomyoma patients was higher, whereas no significant difference in height was observed in leiomyoma versus control group. The BMI in leiomyoma group was significantly high, indicating 11% increase as compared with controls. Out of 46 women with uterine leiomyoma, 12 (26%) women were obese. Systolic blood pressure, in the current study, varied non-significantly, whereas a significant increase of 6% in diastolic blood pressure was observed in leiomyoma group in relation to controls.

Table 1 :Summary of Clinicopathological patterns of uterine leiomyomas-

Characteristics	Frequency (n)	percentage
Age (Years)		
21-30	10	21.7
31-40	18	39.1
41-50	17	37.0
>50	1	2.2
Classification of leiomyoma		
Intramural leiomyomas	28	60.9
Subserosal leiomyomas	13	28.3
Submucosal leiomyomas	1	2.2
Mixed sites	4	8.7
Number of leiomyomas		
Single	24	52.2
Multiple	22	47.8
Menorrhagia		
Present	29	63.0
Absent	17	37.0
Family history of leiomyoma		
Present	12	26.0
Absent	34	74.0
Parity		
Nulliparous	14	30.0
Parous	32	69.0
Weight		
Obese	12	26.0
Non obese	34	74.0
Anemia		
Non anemic	29	63.0
Anemic	17	37
Types of anemia		
Mild anemia	4	24
Moderate anemia	9	53
Severe anemia	4	24

The parity of leiomyoma group was significantly low as compared to controls with 25% reduction ($p=0.03$). Moreover, 14 (30%) leiomyoma women were found nulliparous. Serum electrolyte analysis revealed non-significant increase of 1.4% in serum sodium, a significant decrease of 18% in serum potassium, a marked increase of 9% in serum chloride and a significant elevation of 21% in serum calcium concentration in leiomyoma patients in relation to control subjects. Among marker enzymes, significant elevations in the serum levels of ALT(40%), ACP(22%) and AST (19%) were observed in leiomyoma patients in comparison with controls. However, level of ALP declined non-significantly by 11% in as comparable groups. This scatter diagram shows significant correlation between largest leiomyoma volume and AST level (Table 2.)

Age distribution frequency of fibroid patients exhibited that highest number of fibroid patients were between the age of 31-40 years with 39.1% of the leiomyoma patients, followed by age group of 41-50 years with 37% patients, followed by the age group of 21-30 years with 21.7% of the patients (Figure 1).

Among types of leiomyomas, the most frequent type was intramural (61%) followed by subserosal fibroids (28%). It was observed that incidences of mixed sites (9%) and submucosal leiomyomas (2%) were relatively low (Figure 2).

Table 2: Comparison of anthropometric parameters, age, parity, electrolyte profile and marker enzymes among control and leiomyoma groups. Values are Mean \pm SEM.

Parameters	Control group (n=44)	Leiomyoma group (n=46)	p-value	% Difference
Weight (kg)	57.82 \pm 0.93	64.85 \pm 2.01	0.0024**	12% \uparrow
Height (m)	1.58 \pm 0.01	1.58 \pm 0.01	0.73	0%
BMI (kg/m ²)	23.20 \pm 0.27	25.68 \pm 0.67	0.0012**	11% \uparrow
SBP (mmHg)	115.8 \pm 1.54	119.8 \pm 1.62	0.07	4% \uparrow
DBP (mmHg)	72.61 \pm 1.31	77.07 \pm 1.14	0.012*	6% \uparrow
Parity	4.00 \pm 0.26	3.00 \pm 0.38	0.03*	25% \downarrow
Age	35.32 \pm 1.68	38.83 \pm 1.12	0.07	9% \uparrow
Sodium (mmol/L)	154.8 \pm 3.51	157.1 \pm 3.34	0.64	1.48% \uparrow
Potassium (mmol/L)	5.50 \pm 0.20	1.10 \pm 0.25	0.0027**	20% \downarrow
Chloride (mmol/L)	101.7 \pm 1.24	111.3 \pm 1.82	<0.0001** *	9.43% \uparrow
Calcium (mmol/L)	4.99 \pm 0.13	6.13 \pm 0.10	<0.0001** *	22.8% \uparrow
Alkaline phosphatase (U/L)	228.5 \pm 10.5	204.8 \pm 8.45	0.08	11% \downarrow
Acid phosphatase (U/L)	1.43 \pm 0.07	1.73 \pm 0.11	0.03*	22% \uparrow
AST(U/L)	25.23 \pm 1.40	30.00 \pm 1.49	0.02*	19% \uparrow
ALT (U/L)	23.64 \pm 1.29	33.02 \pm 1.65	0.0001***	40% \uparrow

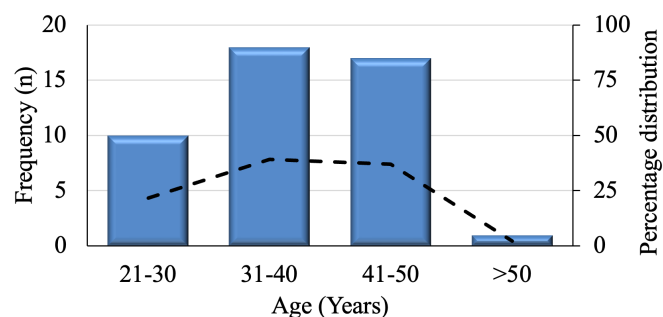


Figure 1: Frequency distribution (primary y-axis) and percentage (secondary y-axis) of the leiomyoma patients grouped according to their age. The highest frequency and percentage were record in the age group of 31-40 years, followed by age group 41-50 years, followed by the age group of 21-30 years.

Regarding the number of leiomyomas, rate of occurrence of single leiomyomas was high with 52% cases, whereas 22 cases with multiple leiomyoma (48%) were identified. Among leiomyoma patients, menorrhagia was widespread with 63% cases, whereas 17 patients (37%) exhibited absence of menorrhagia. Family history of leiomyoma was found in 12 (26%) out of 46 leiomyoma women. According to the blood Hb picture, 29 patients (63%) of leiomyoma were non anemic,

whereas 17 patients (3%) were anemic. Among anemic patient 53% patients had moderate anemia in comparison to mild (24%) and severe anemia (24%) (Figure. 3).

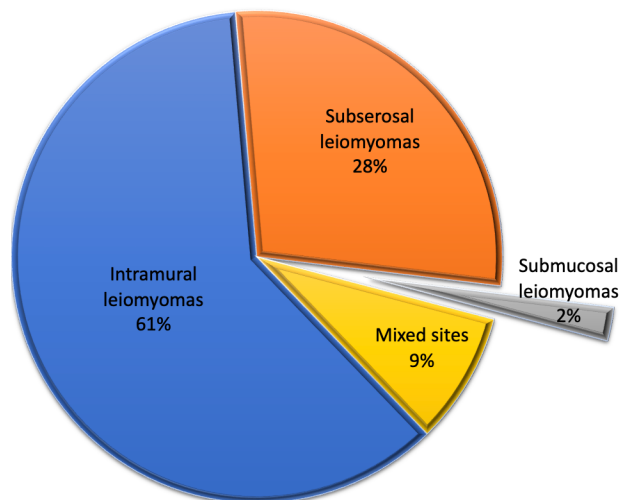


Figure 2: Percentage distribution of different types of uterine leiomyomas. The highest percentage of 61% of intramural leiomyomas were found followed by subserosal leiomyomas 28%, whereas mixed sites and submucosal leiomyomas was relatively low.

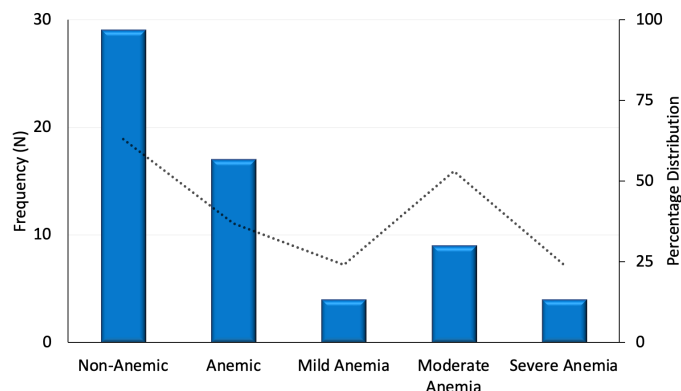


Fig. 3: Frequency and percentage distribution of non-anemic (63%) and anemic (17%) leiomyoma patients. Among anemic patients 53% had moderate anemia whereas 24 percent patients had mild and severe anemia.

Discussion

Our results show that most common leiomyomas were found in age group 31 to 50 years. Risk of uterine fibroids increases with age that may be on the account of collective stimulating effects of progesterone and estrogen on uterine muscular tissues (Bariani et al., 2020; He et al. 2013).

In the current study no significant association was found between uterine leiomyoma risk and body height despite the positive association between plasma follicular phase estradiol level and height. As compared to the controls mean BMI of leiomyoma group was significantly higher. Obesity and increased BMI may influence the leiomyoma risk by decreasing the production of sex hormone binding globulins (SHBG) from hepatic cells which eventually leads to increased availability of

biologically active androgen as well as estrogen in obese women (Maggio et al., 2008). In obese women conversion of androgen to estrogen is also increased by more adipose tissues (Katzner et al., 2021). Moreover, obesity accompany dyslipidemia, hypertension and hyperinsulinemia stimulate uterine fibroid cells by preferentially mediating growth factors (Hoffman et al., 2021; Baird et al., 2009).

A significant increase in diastolic blood pressure was observed in leiomyoma group. Endothelial dysfunction caused by altered secretions of certain vasoactive factors may be responsible for hypertension in leiomyoma patients (Kirschen et al., 2021).

A substantial decrease of parity was found in leiomyoma patients. Nulliparity or reduced parity are most prospective factors for uterine fibroid origin. Nulliparity may contribute to this disease by increasing the stimulation of uterine smooth muscle cells via unchecked progesterone and estrogen production by an ovulatory cycle linked with them (Yang et al., 2022; Marshall et al., 2004;). Alternatively, uterine fibroids may actually contribute to nulliparity through interfering with normal process of ovum implantation and progression of pregnancy by their protrusion into the uterine cavity (Turocy, & Williams, 2022). Intramural myomas were most common in current study because these are frequently the benign tumors of muscular origin, arranged inside the uterine smooth muscle cells in concentric whorl (Sarkodie et al., 2012).

As compared to multiple fibroids single uterine leiomyomas were diagnosed in higher frequency that may be associated to racial or genetic elements (Abdullah & Gomaa, 2013). Menorrhagia was found in majority of the participants. Disturbance in normal menstrual cycle leads to the greater incidence of menorrhagia. This disturbance is caused by altered angiogenesis in the form of abnormal appearance and distribution of blood vessels in uterine fibroid tissues (Dolmans et al., 2021). In present study family history of myoma was found in 26% women. Due to genetic basis of these neoplasms, there is an association between and positive family history of fibroid and development of uterine myoma (Baird et al., 2007). A significant decrease in blood hemoglobin concentration was observed in leiomyoma group. This decrease is because of the hyper menorrhea caused by fibroids that eventually leads to the iron deficiency anemia (Collins et al., 2021).

An increase in serum sodium (Na^+), calcium (Ca^{2+}) and chloride (Cl) concentration were observed in leiomyoma patients. Though, potassium level was significantly lower in myoma group. Higher estrogen level was actually responsible for an increase in sodium concentration. Inside blood $17\text{-}\beta$ estradiol plays an essential role in sodium retention. It retains sodium either directly by stimulation of renal tubules receptors or indirectly via aldosterone (Boschitsch et al., 2010). In kidneys aldosterone receptors over simulated by elevated estrogen level which eventually enhance the physiological processes of aldosterone e.g., excretion of magnesium and potassium through urine while retention of H_2O along with sodium (Armaninet al., 2018). Additionally, estrogen stimulates potassium release directly in urine in exchange for sodium (Williams, 2005). A noteworthy increase in chloride concentration in serum of leiomyoma patients was due to the higher level of blood sodium by kidney dysfunction and estrogen (Shenget al., 2020). As compared to normal myometrial tissues uterine fibroids cells

manifest high expression of PTH-rP (parathyroid hormone related peptide) genes. This enhanced expression is responsible for an increase in serum calcium (Ca^{2+}) levels in patients because PTH-rP secreted in small amounts from many cells, mostly of squamous origin, decreases calcium excretion via kidney and responsible for hypercalcemia like PTH (Lim & Oliva 2019; Truong et al., 2003).

Concerning marker enzymes, in current study a significant increase in serum acid phosphatase levels was found in women with uterine myomas compared with controls. In benign neoplasms ACP activity may be elevated because of more lysosomal participation for the implementation of apoptosis in order to degenerate these neoplasms (Atkinson, 2018; Zakeri et al., 1994). A non-significant difference was found regarding serum alkaline phosphatase levels, in current study between comparable groups. This indicates ALP as non-predictive of uterine myomas.

Moreover, Circulating ALT and AST were significantly higher in leiomyoma patients. These elevations are due to enhanced rate of aerobic glycolysis. Actually, glycolysis increases the production of alanine and glutamate that enhanced the aminotransferases activity in tumors tissues than normal tissues (Yoo et al., 2020; Chougule et al., 2008). In this study a significant correlation between the leiomyoma size and serum aspartate transaminase level was also revealed. This finding suggests that the patients with higher serum levels of aspartate transaminase (AST) tend to have larger fibroids.

Conclusion

Taken together the findings of this study, we can conclude that leiomyomas manifest variations in clinical and pathological presentations like other neoplasms with associated complications like infertility, high body mass index (BMI), anemia and menorrhagia in the reproductive women. Changes in the serum electrolyte profiles through estrogen can be related to leiomyomas developments and ACP, ALT and AST levels can be potential predictor marks for leiomyomas besides, the family history as an influencing factor for their development.

Author contributions

The authors confirm the contribution of all authors in the paper NR, SA²: study conception and design; SA¹, AA, HA: sample, data collection and analysis; SA¹, AA: interpretation of results; SA¹, AA, HA; draft manuscript preparation: SA¹, HA, NR. All authors reviewed the results and approved the final version of the manuscript.

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