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ASSOCIATION OF LEUCINE-RICH ALPHA-2-GLYCOPROTEIN 1 WITH HUMAN UTERINE LEIOMYOMA

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Abstract

Background: Uterine leiomyoma, also known as fibroids, is a common benign tumor that affects women of reproductive age. The pathogenesis of uterine leiomyoma involves various molecular and hormonal factors. Leucine-rich α2-glycoprotein 1 (LRG1) and afamin have emerged as potential biomarkers with potential roles in the development and progression of uterine leiomyoma.

Objective: This study aimed to investigate the correlation of serum levels of LRG1 with uterine leiomyoma and assess their potential as biomarkers for the condition.

Methodology: A case-control study was conducted, including 63 cases with uterine leiomyoma and 30 controls without the condition. The participants' demographic data, including age, marital status, occupation, body mass index (BMI), and parity status, were collected. Serum levels of LRG1 were measured using enzyme-linked immunosorbent assays. The size of fibroids was assessed through imaging techniques.

Results: The mean age of the cases and controls was 39.52±6.64 and 37.80±5.89 years, respectively. Among cases 63(100%) and controls 30(100%) all were married. None of them were unmarried. The mean BMI of case and controls was 23.34±3.89 and 23.35±1.76 respectively. The most commonly reported symptom was heavy per vaginal bleeding, which was observed in 30 cases, accounting for 47.62% of the total cases. Parity status of cases showed that 13(20.6%) cases were multiparous, 15(23.8%) were primi-parous and 35(%) were multiparous Mean fibroids size among cases was 26.72±23.15. Among cases mean Leucine rich α2-glycoprotein 1 level was higher as compared to controls.

Conclusion: This study suggests that serum levels of LRG1 serve as reliable diagnostic markers for uterine leiomyoma. These findings contribute to the existing knowledge about the pathophysiology of uterine leiomyoma and highlight the potential involvement of LRG1 in the progression of this condition.

Keywords: Leucine-rich α2-glycoprotein 1; Uterine leiomyoma

Introduction

The female reproductive system's most frequent solid tumor, leiomyoma affects 20–40 percent of women. For example, myomas are more common during pregnancy, which is characterised by hyperestrogenism, and they are less common during menopause, which is characterized by estrogenic deficiency (Pavone *et al*., 2018). Because of its association with infertility and poor implantation rates during IVF therapy, uterine myomatosis in pregnancy has a prevalence of 0.1–3.9 percent, according to epidemiologic study (Kaganov *et al*., 2018). Uterine fibromyomas have been linked to an increased risk of spontaneous miscarriage, preterm labor, placental abruption, premature rupture of the membranes (PROM), fetal malpresentation, labor dystocia, caesarean delivery, postpartum hemorrhage, and hysterectomy, despite the fact that many women with uterine fibroids successfully complete their pregnancies. It is therefore necessary to use particular attention when diagnosing uterine fibromatosis during pregnancy in order to make the optimal management decision (Lakhman *et al*., 2017).

The most frequent benign pelvic tumor in women is uterine leiomyomas. They're monoclonal tumors of the myometrium's smooth muscle cells, and they're made up of a lot of extracellular matrix, which includes collagen, fibronectin, and proteoglycan. The tumors are typically encircled by a thin pseudo capsule made up of areolar tissue and compressed muscle fibres (Afrin *et al*., 2021). Leiomyomas can develop to the point where they alter the shape of the uterine cavity or its surface. Despite their benign nature, they usually induce severe symptoms like heavy, irregular, and protracted menstrual flow, as well as anemia (Vilos *et al*., 2015). Uterine leiomyomas have also been associated to a range of other medical issues, including infertility, recurrent abortion, and preterm deliveries. Women's health is substantially impacted by various clinical conditions. Uterine leiomyomas are the most commonly reported reason for more than 600,000 hysterectomies performed in the United States each year, and this major surgery is associated with significant morbidity and death, as well as a $2.2 billion yearly economic burden on health-care delivery systems (Wu *et al*., 2011). Uterine leiomyomas are benign tumors that affect up to 60 percent to 80 percent of women in their reproductive years. Although most leiomyomas are asymptomatic, up to 20 percent of them could induce menorrhagia, pelvic pain, and genitourinary symptoms. Leiomyomas form in the presence of gonadal steroids, attack women of African descent disproportionately, and are associated to 10 percent of infertility cases (Santos & Cunha, 2015). Radiographic modalities or surgical procedures are routinely employed to diagnose uterine fibroids. The sensitivity and specificity of existing diagnostic procedures varies; they now range from 50 percent to 20 percent for a hysterosalpingogram (HSG), 90 percent to 87 percent for transvaginal ultrasound, and around 100 percent for magnetic resonance imaging (MRI) (D'Angelo & Prat, 2010).

Despite the fact that the specific cause of uterine fibroids is unknown, research and clinical experience have pointed to a variety of potential explanations. Many tumorigenic muscle cells in fibroids have had their genes changed in ways that aren't observed in normal uterine muscle cells (Toro *et al*., 2006). It appears that the hormones progesterone and estrogen, which are responsible for promoting the growth of fibroids by proliferating uterine lining formation called endometrium throughout every menstrual cycle for pregnancy preparation, are also responsible for proliferating uterine lining formation called endometrium (Reynolds, 2007). Fibroids have much more oestrogen and progesterone receptors than normal uterine muscle cells, according to research. Fibroids tend to shrink as hormone synthesis declines after menopause. Insulin-like growth factor and other substances that help the body maintain tissues may have an effect on the creation of fibroid tumors (Freytag *et al*., 2021). Extracellular matrix (ECM) is the material that causes cells to attach to one another, similar to how mortar holds bricks together. Fibroids have a higher concentration of ECM, making them fibrous in character. In the cells that make up the fibroid's body, the ECM also stores growth substances and causes physiological changes (Ren *et al*., 2019).

Objective

This study aimed to investigate the correlation of serum levels of LRG1 with uterine leiomyoma and assess their potential as biomarkers for the condition.

Materials and Methods

Samples of uterine fibroid-positive patients were collected from gynecology wards from different hospitals of Sahiwal and controls from normal healthy women without uterine fibroid. All the selected patients were screened at the University of Lahore. The study was conducted as a cross-sectional study. Two groups were taken for analysis. Group A consisted of 63 patients diagnosed with uterine fibroids, while Group B comprised 30 women from the normal population serving as the control group. Blood samples were collected from both groups and two tests, namely leucine-rich α2-glycoprotein 1 were performed on the samples. The sampling technique was used non-probable and convenient.

Inclusion Criteria:

- Women with pre-menopausal age range between 25-50 years.
- Women with uterine leiomyoma with ultrasonography report or biopsy.

Exclusion Criteria:

- Women of ages below 25 and above 50
- Cases of prolactinoma, acromegaly, hypothyroidism, chronic renal insufficiency, and severe liver disease
- Patients using dopamine antagonists like phenothiazines, haloperidol, reserpine, and methyldopa)
- Patients on intravenous cimetidine, verapamil, and monoamine oxidase inhibitors
- Patients of leukemia, lymphoma, ischemic heart disease, meningitis, encephalitis, and pancreatitis.

Data collection:

A questionnaire based on the patient's name, age, parity, BMI, symptoms, signs, blood tests, and ultrasonography report, along with a consent form, was provided to all women with uterine leiomyoma and without uterine leiomyoma. Data were collected through a pre-designed proforma. The first part of the questionnaire contained socio-demographic variables, which were completed during the encounters with the patients. Information regarding the study variables was collected after performing the laboratory work. Consent was taken to draw a blood sample. The blood sample was drawn by standard sampling tubes, centrifuge to isolate serum, and frozen at -20°C for analysis (Nagata, 2019).

Leucine-rich α2-glycoprotein 1 estimation:

Commercially available enzyme-linked immunosorbent assay (ELISA) kits was used to quantify the serum concentrations of human LRG1 (Kamalipooya *et al*., 2021). Ensure that all reagents, standard solutions, and samples are prepared according to the instructions provided. Allow all reagents to reach room temperature before use. The assay should be performed at room temperature. Determine the number of strips needed for the assay and insert them into the frames. Unused strips can be stored at 2-8°C for up to one month. For the blank control, only add substrate solution A, substrate solution B, and Stop solution. Add 50 µl of diluted standard to the standard well, add 50 µl of the sample (recommended dilution: 2-5 times, if necessary) to the sample well, and add 50 µl of biotinylated antigen to each well. Thoroughly mix the contents. Cover the plate with a sealer and incubate for 60 minutes at 37°C. Remove the sealer and liquid from the wells, and manually wash the plate five times with 300 µl of wash buffer. Invert the plate each time, decanting the contents and tapping it 4-5 times on an absorbent material to ensure complete removal of liquid. For automated washing, aspirate all wells and wash five times with wash buffer, slightly overfilling the wells. Blot the plate on an absorbent material. Add 50 µl of avidin-HRP to both the standard and sample wells. Cover the plate with a sealer and incubate for 60 minutes at 37°C. Remove the sealer and wash the plate as described above. Add 50 µl of substrate solution A to each well, followed by adding 50 µl of substrate solution B to each well. Incubate the plate, covered with a new sealer, for 10 minutes at 37°C in the dark. Add 50 µl of Stop Solution to each well, which will cause the blue color to immediately change to yellow. Measure the optical density (OD value) of each well using a microplate reader set to 450 nm within

10 minutes after adding the stop solution.

Statistical Analysis:
Statistical analysis was perform using IBM SPSS Statistics 26.0. An Independent t-test was applied to compare the levels of each biomarker in the serum between the case and control. A *P* value <0.05 was considered indicative of statistical significance.

Results
We included 63 cases and 30 controls based on inclusion and exclusion criteria. Mean age of cases and controls was 39.52±6.64 and 37.80±5.89 years respectively. Among cases 63(100%) and controls 30(100%) all were married. None of them were unmarried. Among cases 2(3.2%) individuals were employed and the remaining 61(96.8%) were unemployed while among controls 17(56.7%) were employed and 13(43.3%) were unemployed. Mean body mass index of case and controls was 23.34±3.89 and 23.35±1.76 respectively. Among cases minimum and maximum BMI was 18 and 29 while among controls it was 19.70 and 27 respectively. Parity status of cases showed that 13(20.6%) cases were multiparous, 15(23.8%) were primi- parous and 35(%) were multiparous while among controls all women were multiparous. Mean fibroids size among cases was 26.72±23.15. Minimum and maximum fibroid size among cases was 2 and 92.82 respectively.

Table 01: Demographic data of patients

Characteristics	Cases (n=63)	Controls (n=30)
Age (Mean ± SD, years)	39.52 ± 6.64	37.80 ± 5.89
Marital Status (%)	All married (100%)	All married (100%)
Employment Status (%)	Employed: 2 (3.2%) Unemployed: 61 (96.8%)	Employed: 17 (56.7%) Unemployed: 13 (43.3%)
Body Mass Index (Mean ± SD)	23.34 ± 3.89	23.35 ± 1.76
BMI Range (Minimum - Maximum)	18 - 29	19.70 - 27
Parity Status (%)	Multiparous: 13 (20.6%) Primi-parous: 15 (23.8%) Multiparous: 35 (%)	All multiparous (100%)
Mean Fibroid Size ± SD	26.72 ± 23.15	-
Fibroid Size Range (Min - Max)	2 - 92.82	-

Table 2 presents the symptoms reported among the cases. The table displays the frequency and percentage of each symptom. The most commonly reported symptom was heavy per vaginal bleeding, which was observed in 30 cases, accounting for 47.62% of the total cases. Anemia was the second most frequent symptom, reported in 20 cases, representing 31.75% of the cases. Other symptoms included heavy menstrual bleeding, primary infertility, lower abdomen pain, dysmenorrhea (painful menstruation), secondary infertility, irregular menstrual cycle, and heavy bleeding. Each of these symptoms was reported in varying frequencies, ranging from 2 to 8 cases, and their percentages ranged from 3.17% to 12.70%.

Table 2: Symptoms among Cases

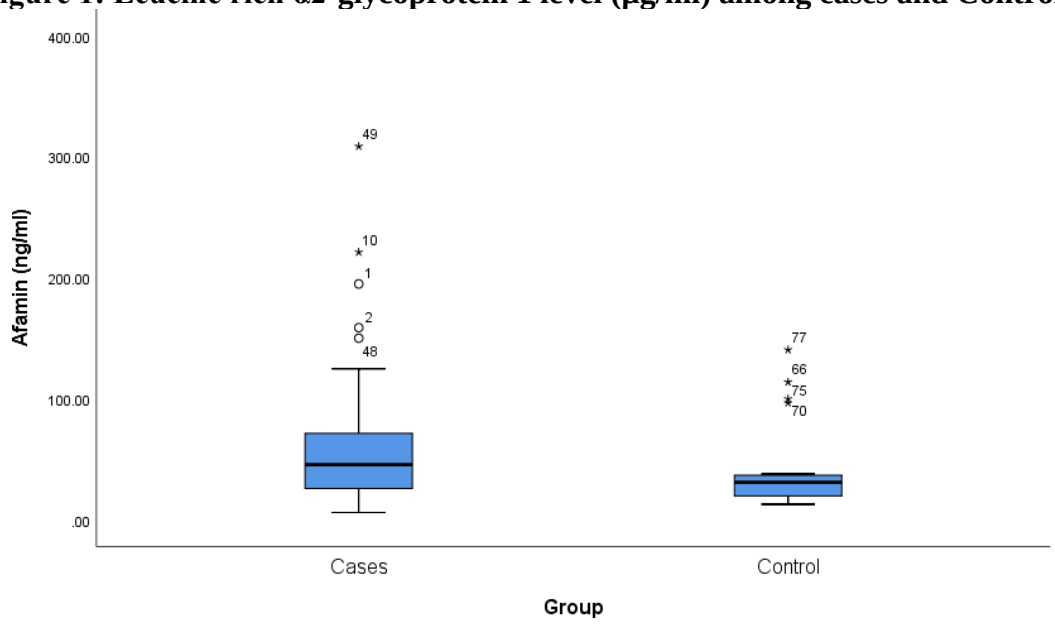
	Frequency	**Percent**
Heavy per vaginal Bleeding	30	47.62%
Anemia	20	31.75%
Heavy menstrual bleeding	8	12.70%
Primary Infertility	8	12.70%
Lower abdomen pain	7	11.11%
Dysmenorrhea	5	7.94%
Secondary infertility	3	4.76%
Irregular menstrual cycle	3	4.76%
Heavy bleeding	2	3.17%

Among cases mean Leucine rich α2-glycoprotein 1 level was higher as compared to controls. However, Leucine rich α2-glycoprotein 1 level show statistically significant difference among case and controls.

Table 3: Leucine rich α2-glycoprotein 1 level (µg/ml) among cases and Controls

	Cases	**Controls**
n	**63**	**30**
Mean	4.64	2.88
SD	1.03	1.20
Minimum	2.19	1.36
Maximum	6.66	3.78
p-value	**0.003**	

Figure 1: Leucine rich α2-glycoprotein 1 level (µg/ml) among cases and Controls.



The box plot shows Leucine rich α2-glycoprotein1 level among cases and controls. It is quite evident that cases had higher value of Leucine rich α2-glycoprotein 1 level as compared to controls.

Discussion

Uterine leiomyomas, also known as uterine fibroids, are benign tumors that develop in the smooth muscle layer of the uterus. They are one of the most common gynecological conditions affecting women of reproductive age. However, the correlation between serum levels of specific proteins, such as LRG1 and afamin, with uterine leiomyoma is not well-established, as of my knowledge cutoff in

September 2021 (Wierman, 2021). LRG1 is a glycoprotein involved in various biological processes, including inflammation, angiogenesis, and tumor progression. Some studies have suggested that LRG1 may play a role in the development and progression of certain cancers, such as colorectal cancer, lung cancer, and hepatocellular carcinoma (Roopashri *et al*., 2023). However, the specific association between LRG1 and uterine leiomyoma has not been extensively investigated. Afamin, also known as alpha-albumin or vitamin E-binding protein, is primarily produced by the liver and is involved in transporting vitamin E in the bloodstream. It has been studied in relation to various health conditions, including pregnancy complications, cardiovascular disease, and cancer. However, its correlation with uterine leiomyoma has not been extensively explored (Andersen, Boylan, Xue, *et al*., 2010).

Uterine leiomyomas, commonly known as fibroids, represent the most prevalent benign tumors affecting women of reproductive age worldwide. These smooth muscle tumors of the uterus often result in significant morbidity, leading to symptoms such as abnormal bleeding, pelvic pain, and reproductive dysfunction. Despite their clinical significance, the precise molecular mechanisms underlying the development and progression of uterine leiomyomas remain incompletely understood. Leucine-Rich Alpha-2-Glycoprotein 1 (LRG1), a glycoprotein recently implicated in various physiological and pathological processes, has garnered attention due to its role in angiogenesis, inflammation, and tissue remodeling. Studies have suggested its involvement in the regulation of vascular homeostasis and its contribution to pathological conditions marked by aberrant angiogenesis. Notably, aberrations in angiogenesis have been proposed as potential drivers in the pathogenesis of uterine leiomyomas.

While the etiology of uterine leiomyomas is multifactorial, exploring the potential association between LRG1 and the pathogenesis of these tumors presents a promising avenue. Understanding the involvement of LRG1 in uterine leiomyomas may offer insights into the molecular mechanisms underpinning their growth, potential novel diagnostic markers, and targeted therapeutic interventions.

Conclusion

This study suggests that serum levels of LRG1 serve as reliable diagnostic markers for uterine leiomyoma. These findings contribute to the existing knowledge about the pathophysiology of uterine leiomyoma and highlight.

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