ORIGINAL ARTICLE

Serum Albumin and C3 Complement Levels in Endometriosis

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ABSTRACT

Objective: To compare the efficacy of albumin and C3 complement as serum marker for the early diagnosis of endometriosis in women of reproductive age.

Study Design: Cross-sectional comparative study.

Place and Duration of Study: Biochemistry Department, Islamic International Medical College, Rawalpindi, in collaboration with the Gynecology and Obstetrics Department, Pakistan Railways Hospital, Rawalpindi, from March 2017 to February 2018.

Methodology: Forty-four patients with endometriosis and 44 controls of reproductive age group were enrolled for the study. Those having other comorbid systemic or endocrine diseases were excluded from the study. Levels of albumin and C3 complement were measured by using Micro Lab 300 and ELISA, respectively; and the results were compared by using SPSS version 21.

Results: The mean serum albumin levels were 4.62 ±0.88 g/dl and 4.42 ±0.52 g/dl in the control and endometriosis groups, respectively (p=0.201). The mean serum C3 levels were 1120.9 ±265 µg/ml and 2241.0 ±293 µg/ml in the control and endometriosis patient groups, respectively (p <0.001).

Conclusion: Serum C3 complement were significantly increased in endometriosis patients but not in controls; whereas, serum albumin levels were not significantly different in endometriosis cases as compared to the controls.

Key Words: Albumin, C3 complement, Endometriosis.

INTRODUCTION

Endometriosis is defined by the growth of benign endometrial tissue outside the endometrium of uterus. It occurs commonly in women of reproductive age.[1] Investigations done on females between 18 to 45 years of ages in Europe showed the prevalence of hospitalisation for endometriosis as 0.9%.[2] In the Pakistani population, approximately 33% women suffering from chronic pelvic pain and 10% young girls with dysmenorrhea have been diagnosed by laparoscopy for endometriosis. Various factors have been proposed to play a role in the establishment and development of endometriosis. These include inflammation, hormonal activity, menstrual cycle pattern, organic chlorine burden, prostaglandin metabolism, and immunological factors.[3] The specific genes responsible for this ailment are an area of active investigation, and monozygotic twin research studies show high concordance rates both in histological terms and disease stage.[4]

The progression of endometriosis is very much related to oxidative stress. The relation between endometriosis and the reactive oxygen species (ROS) production is widely accepted and intensely studied.[5] Endometriosis is a hormone-dependent disease, because its symptoms

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are usually limited to the reproductive period and also respond to hormonal treatment. The main symptoms include dysmenorrhea, deep dyspareunia, infertility, tenesmus, and dysuria. Abnormal uterine contractions patterns are mainly related with medical entities like dysmenorrhea, abnormal transport of gametes and implantation and infertility.[6]

In women with clinical doubt of endometriosis, diagnostic laparoscopy followed by histopathological examination can be used to confirm the diagnosis in 78% to 84% of the patients.[7,8] Clinically, an ideal test is required with high sensitivity that does not miss any individuals with endometriosis or other pelvic conditions that might benefit from early diagnostic or operative laparoscopy.[9]

This study was done to evaluate the diagnostic value of albumin and C3 complement markers for the early diagnosis of endometriosis.

METHODOLOGY

This study was held from March 2017 to Feb 2018 and included a total of 88 females. The patients were divided into two groups; i.e. 44 cases and 44 controls. The study was carried out at the Biochemistry Department, Islamic International Medical College Rawalpindi, in collaboration with Department of Gynecology and Obstetrics, Pakistan Railways Hospital, Rawalpindi. A formal approval was obtained from the Institutional Ethical Review Committee of Islamic International Medical College before the commencement of the study. The inclusion criteria for endometriosis patients was of reproductive age females between 18 and 45 years who were diagnosed for endo-

metriosis on laparoscopy, presence of non-resolving hemorrhagic cysts on ultrasonography, and patients symptomatology. Patients presenting to the treating physician in the Medical Outpatient Department, Pakistan Railways Hospital were selected for the study. Those having history of any malignancy, pregnancy or other comorbid systemic or endocrine diseases were excluded from the study.

Informed verbal and written consents from the patients were taken and their demographic data were recorded on a proforma by the investigators. The patients were divided into two groups, group I having controls and group II having endometriosis patients. Random blood sample was taken and 5 ml of blood was collected from the median cubital vein. The blood samples were centrifuged for 15 minutes at 1000 rpm for serum separation then the separated serum was transferred to eppendorf tubes at -80°C in the biochemistry laboratory for further analysis. Serum C3 complement levels were determined using an ELISA kit from bioassay technology and following the instructions of the manufacturer. The C3 levels between 900 to 1800 µg/ml were considered as normal. The serum albumin level of the study population was checked by calorimetric method on photometric system. The kit that was used was manufactured by Merck Private Limited, France. Reference values for serum albumin were between 4.8 to 5.5 g per dL.

The collected data was entered in the SPSS version 21 for analysis. Descriptive statistics was applied to measure frequency of categorical variables and mean with standard deviations for continuous variables like age, BMI, pain severity, and menstrual cycle. Comparisons between controls and patients groups were performed using independent t-test; and p-value less than 0.05 was considered as significant.

RESULTS

A total of 88 subjects were included in the study, consisting of 44 controls in group I and 44 endometriosis cases in group II. The mean ages of controls and cases were 35.14 ±6.92 and 34.64 ±9.43 years, respectively (Table I). The mean weights of controls and cases were 59.02 ±9.35 and 57.84 ±7.08 Kg, respectively. The mean BMIs of controls and cases were 23.13 ±2.03 and 22.69 ±3.29 Kg/m², respectively. The mean serum albumin levels were 4.62 ±0.88 g/dl and 4.42 ±0.52 g/dl in the control and endometriosis groups, respectively (Table II). There was no significant difference in values of serum albumin in the two groups (p value=0.201). The mean serum C3 levels were 1120.9 ±265 (µg/ml) and 2241.0 ±293 µg/ml in the control and endometriosis patients groups, respectively (Table II). The difference in the serum C3 levels were significant between these two groups (p <0.001).

Table I: General characteristics of patients with and without endometriosis.

N=88	Controls (N=44)	Endometriosis patients (N= 44)	p-value
Age (years)	35.1 ±6.92	34.6 ±9.43	0.778
Weight (kg)	59.02 ±9.35	57.84 ±7.08	0.506
BMI Kg/m²	23.13 ±2.03	22.69 ±3.29	0.450

Table II: Serum albumin and C3 complement levels.

	Controls	Endometriosis patients	p-value
Serum albumin level (g/dL)	4.62 ±0.88	4.42 ±0.52	0.201
Serum C3 level (µg/ml)	1120.9 ±265	2241.0 ±293	<0.001

DISCUSSION

Diagnosing endometriosis patients without surgery is one of the most important tasks in gynecology. This would permit to identify endometriosis in patients in a short window of time after initial symptoms appear, thus avoiding a substantial number of unnecessary diagnostic procedures.[10] In fact, an effective non-invasive diagnostic test for endometriosis would allow to identify patients for laparoscopic treatment of endometriosis very early, as early treatment has been proven to improve fertility rate. Furthermore, it may decline disease progression. Previously some studies have shown that non-invasive diagnostic procedure are not affected by the menstrual cycle phase and it is independent from endometriosis stage, thus anticipating that the hormonal cycles and the effects of the disease on the normal physiology of the body do not have any influence on the diagnostic performance.[11]

Many past studies show that mean age of patients suffering from endometriosis was 29 ±4.3 years ranging from 19-40 years. This is in association to other studies which quote low prevalence of endometriosis in either extremes of age and high prevalence in women of reproductive age.[12]

In this study, mean ages of controls and cases were 35.14 ±6.92 and 34.64 ±9.43 years, respectively (Table I). The inverse association between current BMI and endometriosis shows that higher body size reduces an individual's subsequent risk of developing endometriosis.[16] The present study found that there were 41 patients in control group while 44 patients in endometriosis group below BMI of 30 Kg/m². There were three patients in control group while none in endometriosis group above BMI of 30 Kg/m². However, it was also noted that women with endometriosis had lower weight and BMI as compared to control group.

This study, which measured the level of complement components in the serum of women with and without endometriosis, found higher concentrations of C3 in the serum of women with endometriosis compared with

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healthy women. The diagnostic value of marking complement components has been acknowledged for auto immunologic, neurologic and malignant diseases.[13] Further studies on large cohorts of endometriosis patients performed in different institutions are required to confirm the diagnostic value of this assay and to propose it to the scientific community as an effective diagnostic tool for detecting endometriosis. Previous studies have documented the expression and regulation of complement system components in the human endometrium and C3 has also been examined in eutopic and ectopic endometria and found to be increased in both locations in women with endometriosis.[14,15]

In a study, ELISA was performed on a large cohort of endometriosis (n=100) cases and healthy controls to establish the differential expression of C3 and albumin levels. The analyses showed the statistical significant results for both these proteins with p value less than or equal to 0.05.[11]

Serum levels of albumin in control and endometriosis group were found to be not significantly different between the two study groups. Serum albumin levels have been classically thought to reflect the nutritional status of patients. This concept has been challenged in the last two decades as multiple factors, such as inflammation, appeared to affect albumin levels independent of nutrition.[16] Albumin is the most abundant protein in human serum. It is a monomeric molecule present in the interstitial space and body fluids. Liver is the main organ concerned with its production at the rate of 9 to 12 g/dl, and synthesis is chiefly affected by colloid osmotic pressure and inflammatory states, and to a lesser extent by hormones and nutritional status. The catabolism of albumin is still debatable but is hypothesized to take place in the vascular endothelium. Reduced food intake can cause hypoalbuminemia but newer studies have found that albumin concentrations may not change for several weeks, and inflammation was found to reduce albumin concentration in the serum regardless of malnutrition.[17-19] Recently, the role of inflammation and oxidative stress was found in the immune pathogenesis of myasthenia gravis (MG), and it was found that serum albumin may be associated with the development of MG.[18,19]

In one study, levels of serum albumin was studied in myasthenia gravis. All subjects were divided into triple groups by the serum albumin level, in order to derive a deeper understanding of the relationship between S-Alb level and MG severity. Albumin levels were categorised as group I = 2.2 to 3.8 g/dl, group II = 3.5 to 4.5 g/dl, and group III = 4.6 to 4.9 g/dl. Results showed that patients with higher disease severity had a lower serum albumin than those with lower disease severity. Furthermore, it was found that levels of serum albumin in patients with myasthenic crisis were significantly lower compared with those without myasthenic crisis.[20,21] In another study considering normal and hypoalbumenemic, the patients from hypoalbuminemia group, 39.29% patients suffered from higher disease severity *versus* 21.95% from normal albumin group (p=0.016).[22] In another study, ELISA was performed on a large cohort of endometriosis (n=100) cases and healthy controls to establish the differential expression of C3 and albumin levels. The analyses showed the statistical significant results for both these proteins with p value less than or equal to 0.05.[21]

Some studies have found higher values of C3a fragments in infertile females with or without endometriosis.[23] Some other studies have also found increased levels of complement cascade proteins in peritoneal fluid of females suffering from endometriosis.[24] Some authors have identified several other proteins in serum and urine samples like vitamin D binding protein, alpha 1 antitrypsin, pre-albumin and enolase 1, but as individual analytes, these proteins show insufficient sensitivity and specificity to be able to qualify to become non-invasive markers for detecting endometriosis.[24] Likewise, specific antibodies against transferrin, carbonic anhydrase, alpha 2 glycoprotein, serum albumin, low density lipoproteins, and cardiolipin have shown promise as potential biomarkers for endometriosis.[24]

Further studies should be planned to confirm the pattern of expression of several proteins found in a larger cohorts of females and also to further characterise the proteins based on the proteomic approach. We had some strengths of study, as it was first of its kind in Pakistan, regarding the possible role of non-invasive serum markers in detecting endometriosis. It was a cross sectional study to compare the two markers in two equal groups of female with and without endometriosis. However, there were also some limitations in this study as the grades of endometriosis were not taken into account and their possible effect on these markers levels. Advanced research regarding gene sequencing at molecular level is suggested to find the various gene variants in this complex, multifactorial disease. Multidisciplinary approach is also required from all medical departments to manage and diagnose endometriosis with the help of devising non-invasive investigative serum markers.

CONCLUSION

Serum C3 complement is significantly increased in endometriosis patients but not in controls; whereas, serum albumin levels are not significantly different in endometriosis cases.

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