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Retrospective Analysis of CRP and Complement 3 Levels in Endometriosis Patients: Insights from ELISA Assessments

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Abstract

Endometriosis refers to the existence of functional endometrial glands and tissue in locations beyond the uterus. This condition frequently leads to persistent pelvic discomfort, occasionally severe, and inflammation is recognized as a contributing factor in the development of endometriosis. Finding a non-invasive indicator for endometriosis would greatly assist in its early detection and treatment. Therefore, the present study was conducted to study the levels of inflammatory mediators - Complement 3 (C3) and C-reactive protein (CRP) in endometriosis condition. The study was conducted in 40 women who have been laparoscopically diagnosed with endometriosis, and the levels of C3 and serum CRP were estimated in the plasma by ELISA analysis. The results demonstrated significant rise in the levels of serum CRP and C3 in endometriotic women as compared to the levels in non-endometriotic women. Due to the altered regulation of the complement system and the effects of pelvic adhesions and deep infiltrating endometriosis, there is a significant rise in the levels of complement 3 and serum CRP respectively. While immune infiltration and constant inflammatory milieu foster the disease, the biggest pathological consequences arise from aberrant complement activation. Hence, with the aid of pre-clinical and clinical trials, a detailed strategy can be formulated to suppress complement activation and alleviate the devastating effects of endometriosis.

Keywords: *Complement 3, Endometriosis, Health, Inflammatory Mediators, Serum CRP, Well-being.*

Introduction

Endometriosis is defined as the existence of endometrial-like tissue outside the uterus, associated with pelvic pain and infertility in women of the reproductive age group [1]. Laparoscopy remains the most reliable method for diagnosing endometriosis, providing both clinical observations and histological results. Nevertheless, this procedure is intrusive, and numerous patients are hesitant to undergo it as soon as the illness is suspected. Consequently, this reluctance can lead to a delay in diagnosis that extends up to 9 years [2]. While the precise cause of endometriosis remains a subject of debate, Sampson's theory of retrograde menstruation is a commonly acknowledged hypothesis that explains the condition. Moreover, its development is thought to stem from an inflammatory process in the pelvic region that disrupts the proper functioning of immune cells in the peritoneal area. The accurate diagnosis of this condition without resorting to invasive methods continues to pose a significant challenge [1, 3]. Developing a highly accurate biomarker for the non-surgical identification of endometriosis holds the

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potential to enable earlier diagnosis and the prevention of harmful consequences. This objective stands out as a significant area of focus for research [4].

Previous research has suggested that abnormal immune mechanisms and inflammatory reactions play a role in the development and progression of endometriosis [5]. The inflammatory reactions linked to endometriosis rely on heightened activation of macrophages and the cytokines they release in the peritoneal fluid [4]. In light of the various stages in the development of pathogens, numerous studies have aimed to identify a dependable serum marker that could be employed in routine clinical use [2-6].

C-reactive protein (CRP) is a commonly used immune system marker that indicates the presence of infection, inflammation, or tissue injury in standard medical tests. Given that endometriosis is classified as an inflammatory ailment, CRP could potentially serve as an extra option for detecting it non-invasively [6]. The liver produces CRP, an acute phase reactant, in reaction to inflammation. Increased CRP levels in endometriosis may be a sign of inflammation, which has been linked to the severity of the condition or its consequences including pelvic adhesions or deep infiltrating endometriosis [7]. For instance, a study by Abrao et al (1997) discovered that CRP levels were significantly higher in endometriosis patients than in healthy controls [8]. The relationship between CRP levels and endometriosis, however, has been the subject of some research that has produced contradictory findings [9-11].

The complement system constitutes an integral component of the innate immune response. It encompasses a collection of soluble and cell-membrane proteins that collaborate to generate a well-coordinated defensive mechanism for the host and facilitate immune monitoring [12]. Extensive investigations have emphasized that C3 and related complement genes exhibit the highest levels of up-regulation in tissues affected by endometriosis when compared to the normal endometrium [13-15]. The gene expression patterns of both eutopic and ectopic endometrial stromal cells demonstrated elevated expression of transcripts such as C3, C7, and SERPIN5. This indicates that these particular genes are expressed at notably higher levels in these cells [13]. This research study is hence aimed at assessing the levels of CRP and C3 in patients with endometriosis as an opportunity to develop novel therapeutic interventions to reduce the morbidity and mortality of the same. It's important to remember that additional factors, like infections, systemic inflammation, and other medical disorders, might affect CRP and C3 levels and in order to accurately assess endometriosis, a thorough study of the patient's clinical history, physical examination, and other diagnostic findings should be taken into account in addition to CRP and C3 values.

Methodology

Subjects

This study was approved by the Institutional Review Board, and ethical clearance was obtained from the Ethics Committee of Saveetha Medical College & Hospitals, Chennai.

Sample Size

Blood samples were collected from endometriosis patients who visited the Obstetrics and Gynaecology department as out-patients, specifically those between the ages of 35 and 55 and complained of chronic pelvic pain, heavy menstruation and painful intercourse etc., the study also included healthy age-matched controls. Each participant was informed about the study verbally and gave their consent by signing the required paperwork and following relevant guidelines and regulations throughout the research.

Inclusion Criteria

Laparoscopically confirmed endometriosis cases, aged between 35-55 and age-matched healthy controls.

Exclusion Criteria

Known allergy or hypersensitivity, Diabetes, history of liver, or history of kidney disease, hormonal medications, other pelvic inflammatory diseases.

Sample Collection

Morning fasting blood samples were collected from the participants and treated with ethylenediaminetetraacetic acid (EDTA). Following collection, the blood tubes were promptly placed in a refrigerator. To isolate the plasma, the samples were subjected to centrifugation at 3500 rpm for 10 minutes at a temperature of 4°C, all within 15 minutes of the initial collection.

ELISA Analysis

ELISA analysis of complement 3 and c-reactive protein was carried out in the plasma collected from healthy and endometriosis subjects using commercially procured ELISA kits (Ray Biotech Life, USA).

Statistical Analysis

The findings are reported as the average value (mean) along with its associated variability (standard deviation). To assess variances between plasma CRP and Complement 3 concentrations, a comparison was made using either the Mann–Whitney U test or the Kruskal–Wallis test, depending on the circumstances. $P<0.05$ was considered significant. All statistical analyses were carried out using SPSS version 22.0.

Results

Demographic Data

The average age and body mass index of healthy women (with an average age of 35.6 years and an average BMI of 24.2 kg/m^2) were similar to those of women with endometriosis (with an average age of 35.12 years and an average BMI of 24.6 kg/m^2). However, women with endometriosis experienced significantly higher rates of chronic pelvic pain, painful menstruation, painful intercourse, painful defecation, and painful urination compared to the healthy group. This information was gathered based on the responses provided by the participants in the questionnaire.

ELISA Analysis of CRP

A marked ($p<0.001$) increase in the levels of C- C-reactive protein was observed in endometriosis patients compared to that of healthy individuals (Figure 1). The healthy controls exhibited CRP levels of approximately 1.126±0.04mg/dl while the endometriosis patients showed 2.790±0.28mg/dl CRP levels indicating that inflammation plays a pivotal role in the development of endometriosis.

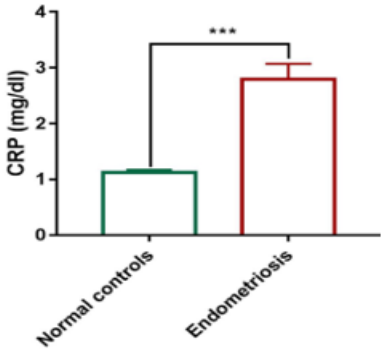

Figure 1. Bar graph showing elevated levels of C-reactive protein levels in endometriosis. For normally distributed continuous variables were described by Mean± SEM and student's t-test were performed. *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs Control.

ELISA Analysis of Complement C3 Levels

The ELISA analysis of C3 was carried out in the plasma samples of endometriosis patients and healthy subjects without endometriosis. The results exhibited significantly elevated ($p<0.001$) C3 levels of 6.110±0.64 in endometriosis patients than that of healthy subjects who exhibited 2.670±0.34, further confirming the involvement of inflammation in endometriosis condition (Figure 2).

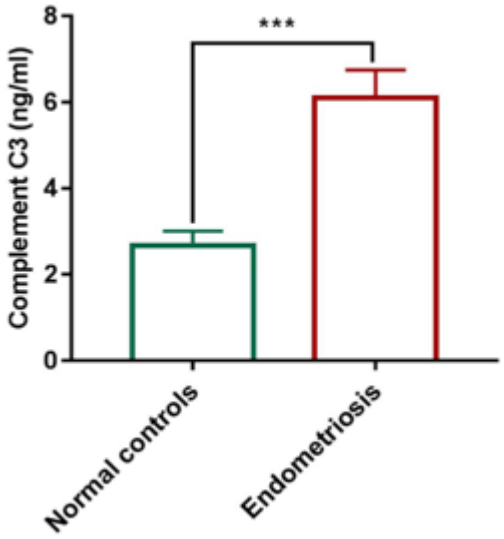

Figure 2. Bar graph showing elevated levels of Complement (C3) levels in endometriosis. Normally distributed continuous variables were described by Mean± SEM and student's t-tests were performed. *p<0.05, **p<0.01, ***p<0.001 vs Control.

Discussion

Endometriosis affects the immune system and stimulates the generation of substances that encourage inflammation [16]. These inflammatory compounds have diverse biological functions, complicating the identification of their specific contribution to inflammation [17,18]. As a result, there remains a persistent desire to monitor the variations in their control within endometriosis, aiming to comprehend the inflammatory mechanism better. In light of this information, the current study discovered higher levels of CRP and C3 in the blood plasma of individuals with endometriosis, whereas healthy individuals did not exhibit an increase in these measured markers of inflammation.

C-reactive protein (CRP), a protein associated with acute inflammatory responses, is commonly utilized as an indicator of existing inflammation in medical settings and is detectable in the majority of labs. Nevertheless, the potential involvement of CRP in endometriosis has been conflicting. Only a few studies have demonstrated increased levels of CRP in the serum or plasma of endometriosis patients [8, 19]. Other studies have reported a significant increase of CRP in the peritoneal fluid while the serum samples did not exhibit any significant changes [9-11]. The discrepancies in results could stem from various factors such as the selection of diverse case groups, variations in the sensitivity of assays, limited statistical strength, improper choice of control subjects, and inadequate consideration of variables that can distort the findings [5]. Our research aligned with previous studies that also found a notable rise in CRP levels. Moreover, the generation of CRP is stimulated by molecules involved in inflammatory signalling, such as IL-1 and IL-6 [19]. This suggests that

the elevated CRP levels observed in our current study imply an activated inflammatory reaction in cases of endometriosis.

The complement system seems to play a central role in driving prolonged inflammation in endometriosis and continues to be a contributing factor in EM-related ovarian cancer [14]. Various components of the complement system, including C3, C4A, C7, factor D, factor B, and factor H, exhibit distinct expression patterns in endometriosis as compared to normal uterine tissues. Additionally, specific genetic variations in the C3 gene have been associated with a higher susceptibility to both endometriosis and the infertility associated with it [20]. Consistent with these findings, our study also indicated notably elevated levels of C3 in patients with endometriosis.

Conclusion

The disease is fuelled by immunological infiltration and a persistent inflammatory environment, but abnormal complement activation is what causes the most severe effects. Therefore, a thorough plan may be developed to decrease complement activation and lessen the debilitating consequences of endometriosis with the use of pre-clinical and clinical research. Further, a multi-target therapy seems to be the most promising approach in the treatment of pain in endometriosis and research should focus on this direction.

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Conflict of Interest

The authors declare no conflict of interest.

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