Original Research Article

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Interleukin-6 and C-Reactive Protein: their association with vitamin D in women with recurrent infections of reproductive system

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Abstract

Aerobic bacteria can colonize the female reproductive system with harmful effects, which may lead to miscarriages, premature deliveries or continue of its growing to cause adverse reproductive systems issues. Increasing in the levels of inflammatory markers may be considered a herald of danger. High vaginal swabs were obtained from 85 women. Of these, 67 patients were suffering recurrent vaginitis and symptoms such as itching, irritation, burning, and vaginal discharged, and 18 apparently healthy controls. Swabs were cultured in a suitable media and the cultivated bacteria were diagnosed in the hospital's laboratory. At the same time of collecting the vaginal swabs, 5 ml of venous blood was withdrawn from the patients and controls. An ELISA method was applied to measure the levels of inflammatory cytokines and concentration of vitamin D. The bacterial growth showed six species of isolated bacteria, which were *Staphylococcus aureus*, *Streptococcus spp.*, *E. coli*, *S.* non *aureus*, *Proteus spp*. and *Klebsiella spp*. The first three species were the most prevalent bacteria, and the serum levels of C reactive protein (CRP) and IL-6 were high in female patients infected with these bacteria. CRP was significantly elevated in sera of the patient's group (P= 0.016), while the increase in IL-6 was not significant. Vitamin D was correlated negatively with IL-6 and positively with CRP, but the correlations did not reach statistical significance. In conclusion, rising of CRP could be an expected result to the bacterial colonizing the reproductive system while IL-6 may develop significantly when the aerobic vaginitis continues until triggering one of the infertility issues.

Keywords: Aerobic vaginitis, IL-6, CRP, Vitamin D, Recurrent infection.

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Introduction

The female's genital tract can be infected with several types of microorganisms. However, bacterial infections are the most common types of these infections. The term vaginitis is used to describe a spectrum of conditions that result in symptoms in the vagina and vulvar such as itching, irritation, burning, odor, and vaginal discharges. Bacterial vaginosis (BV), trichomoniasis and vulvovaginal candidiasis are

the most common reasons for vaginitis[1]. Anaerobic bacteria, such as *Gardnerella vaginalis*, *Atopodium vaginae*, and *Megaphaera spp.* are mostly implicated in causing vaginosis after arising in their prevalence in cervix and vagina against *lactobacilli* prevalence.[2] Amsel criteria, which include evaluation of the four criteria of signs and symptoms are usually used in diagnosis bacterial vaginosis.[1,2] Bacterial vaginosis on several occasions is responsible for the high incidence of diseases such as pelvic inflammatory diseases and endometritis.[3] In addition to its association with miscarriages and preterm birth.[1]

On the other side, various species of commensal bacteria can grow and colonize the female's reproductive system with much evidence supporting their role in infertility. *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus spp.*, and other species of bacteria are currently considered as important causes for infertility.[4,5]

The interaction between BV and proinflammatory cytokines such as Interleukin 6 (IL-6), IL-1, IL-8, or C reactive protein (CRP) have been investigated by several studies.[6,7] These studies attempted to analyze the elements of the interaction between BV and such immunological cytokines, and determine which can be attributed to infertility, abortion, or premature deliveries.[6,7] Other research studies have investigated the relationship between some disorders that cause infertility such as tubal factor infertility (TFI), polycystic ovary syndrome (PCOS) or endometriosis and levels of implicated cytokines.[8-10]

IL-6 is produced by monocytes as a sequence of local infection, and it induces the release of CRP from the liver after its transport to the liver via the bloodstream.[11-13] IL-6 plays a role in adaptive immunity by stimulating humoral immunity and cell-mediated immunity.[12] CRP is a plasma protein, which its concentration increases systematically during an inflammatory response. CRP has important biological and immunological effects by interacting with several effector molecules.[14] After releasing it as a homo-pentameric protein, CRP can irreversibly dissociate at the site of infection into five identical molecules.[15]

Compared to the interesting research dealing with BV and anaerobic bacteria, few sources have been focused on commensal bacteria such as *S. aureus*, *E. coli*, or *Streptococcus spp.*, which can grow and colonize the female's reproductive system, and its role in pathogenicity and infertility. Therefore, the current study aimed to investigate if the levels of inflammatory markers can be affected by infection of female's reproductive system with species of aerobic bacteria, which do not classify as reasons for BV, and to determine the correlation between the levels of these cytokines and concentration of vitamin D.

Materials and Methods

The protocol of the study was reviewed and approved by the Council of College of Science, University of Kerbala (Approval No. 6-1793 in 12.11.2020).

The present research was conducted at the Maternity Teaching Hospital in Karbala. Iraq. A total of 85 women at the age range 18-45 years were enrolled in this research. Of these, 67 women were considered as patients because they have symptoms such as itching, irritation, burning and vaginal discharges and they showed positive bacterial growth culture. In addition, 18 females, apparently healthy were included as controls because they lacked any suggestive symptoms, and their bacterial growth cultures were negative. The women with chlamydial infection were excluded from the study. Also, ladies with any other diseases such as diabetes, rubella, autoimmune diseases, blood and cardiovascular diseases, hormonal disordered, and infectious diseases were also excluded from this study. High vaginal swabs were taken from all females (patients and controls), and all swabs were transferred to the hospital's laboratory to make the bacterium cultures. The diagnoses of bacteria were accomplished by microscopic examination, cultural growth, and biochemical tests.

Serum samples were prepared by collecting 5 ml blood samples from each participant. The blood was placed in gel tubes and centrifuged for 15 minutes at 1006 g. The separated sera were transferred to a new and clean tubes and

kept at -20 °C until used. The levels of IL-6, CRP and vitamin D were measured in the serum samples by using the ELISA procedure. An ELISA kit (Elabscience human IL-6 (Interleukin 6) ELISA kit, USA) with a sensitivity of 4.69 pg/ml was used for IL-6 detection, according to the manufacturer's instructions. An ELISA kit (Elabscience human CRP (C-Reactive Protein) ELISA kit, USA) with a sensitivity of 0.23 ng/ml was used for CRP detection, according to the manufacturer's instructions. An ELISA kit (25-OH vitamin D total (vit D-Direct) Test System, ELISA microwells, Monobind Inc. USA) was used for evaluation vitamin D levels, according to the manufacturer's instructions. The raw data were analyzed statistically by the GraphPad prism software and *P* values under 0.05 were considered statistically significant.

Results

In the present study, six species of bacteria were detected, which were distributed to 38 isolates for *S. aureus*, 10 for *Streptococcus spp.*, 8 for *E. coli*, 7 for *S.* non *aureus*, 2 for *Proteus spp*. and 2 for *Klebsiella spp*. The bacterium which was recorded with few isolates (less than 6) were excluded from any statistical analysis.

There was no statistically significant difference the level of IL-6 in the patients and controls. There was a difference in the mean values between the two groups, but it did not reach statistical significance (Figure 1A). The quantitative values for IL-6 were distributed and calculated according to the type of bacteria, which was recorded to each patient. Although there was no statistical significance, the bacterium *E. coli* showed the highest mean for all registered bacteria, and it was followed by *S. aureus* (Figure 1B).

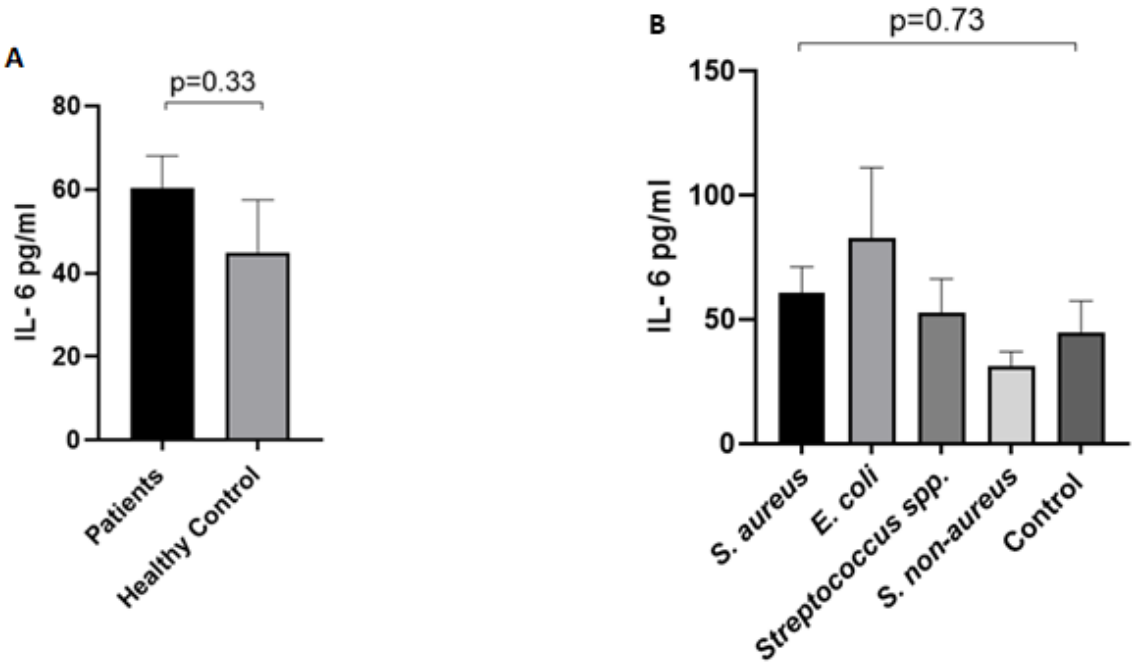

Figure 1. Concentration of IL6 in the study patients and controls. A: The concentration of IL-6 in patients and in healthy control, there were no significant differences after analyzing data by Mann-Whitney test, mean= 60.39, 44.91, SEM=7.73, 12.61, rang= 307.1, 128.4 respectively. B: Concentration of IL-6 according to type of registered bacteria, which were *S. aureus*, *E. coli*, *Streptococcus spp.,* and *S.* non *aureus*, mean= 60.70, 82.70, 52.94, 31.27, SEM=10.63, 28.46, 13.56, 5.78, Range= 307.1, 217.7, 125.8, 25.38 respectively. There were no significant differences after analyzing data by Kruskal-Wallis test.

CRP was measured for the patients and control groups. The patient's group showed a significantly elevated level of CRP compared to the control group (Figure 2A). The concentration values for CRP were also distributed into four categories depending on the type of bacteria, which were registered for the patients. The four categories of CRP levels were compared to the controls, and there was significant differences among all groups (Figure 2B).

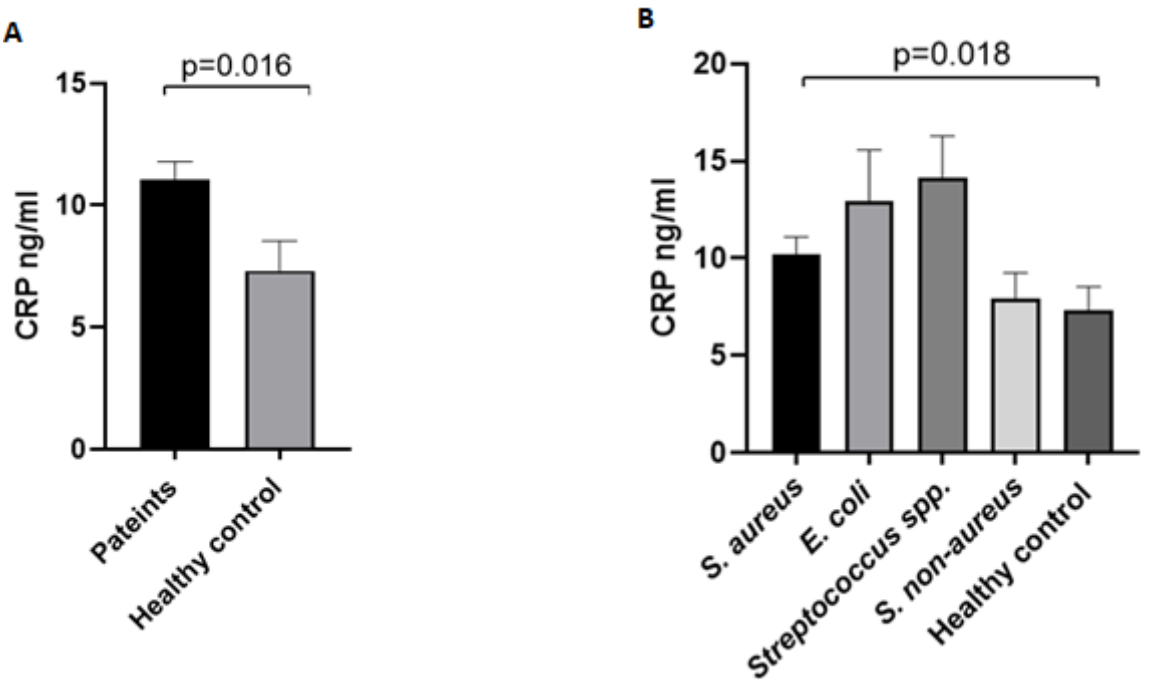

Figure 2. Concentration of CRP in study patients and control subjects. A: The concentration of CRP in patients and healthy control. Significant differences were found in the concentration of CRP in patients and in healthy control after analyzing data by using t-test, mean= 11.06, 7.32, SEM= 0.72, 1.22, range= 26.65, 18.43 respectively. B: Concentration of CRP according to type of registered bacteria, which were *S. aureus*, *E. coli*, *Streptococcus spp.,* and *S.* non *aureus*, there were significant differences between all groups after analyzing data by one-way ANOVA, mean= 10.23, 12.94, 14.16, 7.94, SEM= 0.87, 2.63, 2.14, 1.32, range= 22.86, 22.10, 19.37, 9.34, respectively.

The current research also investigated the relationship between the serum concentration of vitamin D and infection. The data did not show a significant difference between the patients and the control groups and the mean of concentration for the two groups was roughly equal (Figure 3A). The correlation between vitamin D and the two inflammatory factors (CRP and IL-6) were analyzed (Figure 3 B, C). The correlation between vitamin D and IL-6 was inverse, although it was very weak and insignificant. Also, the correlation between vitamin D and CRP was very weak and insignificant.

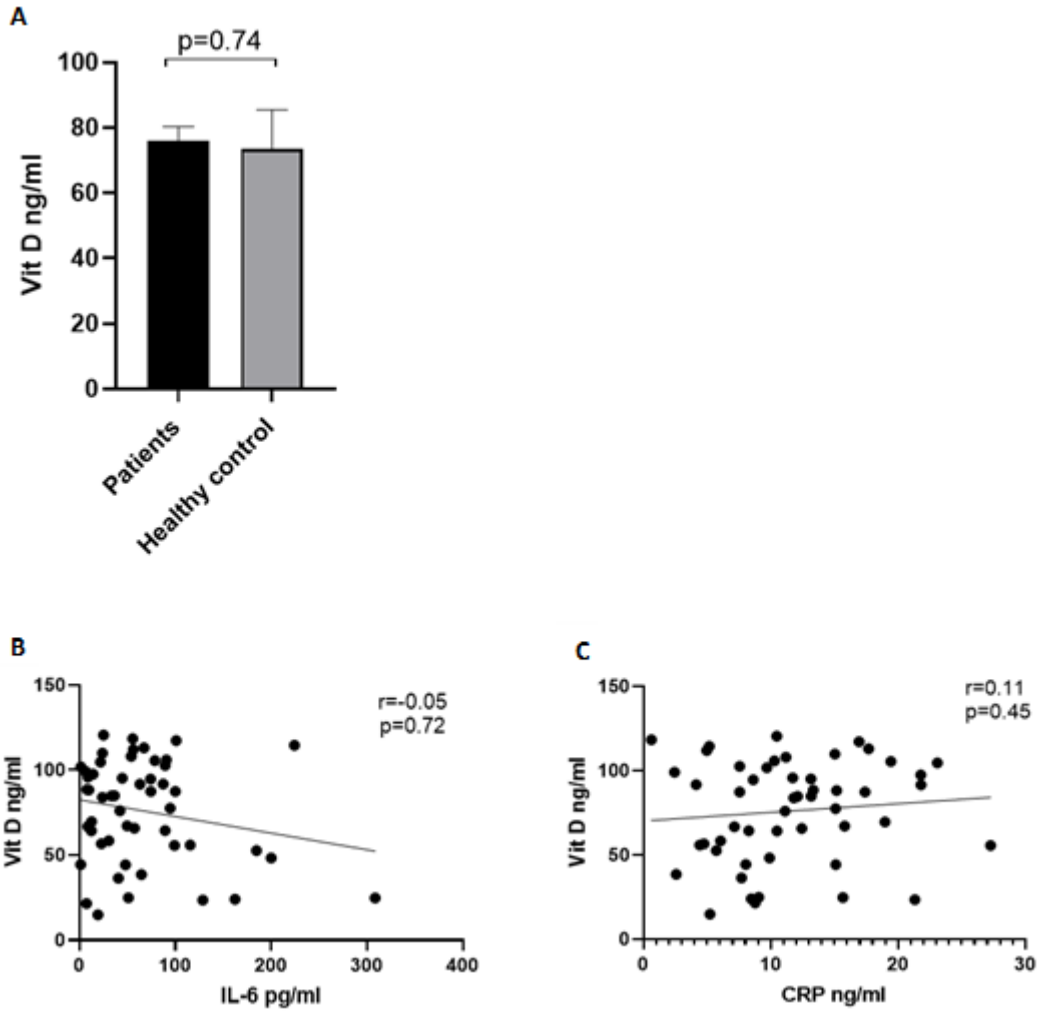

Figure 3. Concentration of vitamin D in study patients and control subjects and its correlation with IL6 and CRP. A: The concentration of vitamin D in patients and controls. The statistical analysis did not show a significant difference after analyzing data by Mann-Whitney test, mean= 76.13, 73.53, SEM= 4.198, 11.90, range= 105.7, 124.1 respectively. B: The correlation between vitamin D and IL-6. There was an inverse relationship without statistically significant was appear after applying spearman correlation. C. the correlation between vitamin D and CRP, spearman correlation was very weak.

Discussion

Blood concentrations of two inflammatory markers (CRP and IL-6) were analyzed to determine their concentration during infection of the female's reproductive system with some species of bacteria. Although there was a difference in the mean of concentration for both markers between the sera of patients and controls, CRP was only significantly elevated. The concentration of both markers was high in sera of patients mainly infected with the species of bacteria *S. aureus*, *E. coli*, and *Streptococcus spp.* and that has appeared significantly with CRP. The patients infected with *E. coli*, and *Streptococcus spp.* showed the highest concentration of CRP among all groups.

The interconnection between the level of cytokines in females with BV has been investigated in much research, some of which tried to connect the high levels of these cytokines to several disorders. In a study comprised 322 women, who were divided into two groups, the chlamydia group and non-chlamydia group, the clinical parameters such as erythrocyte sedimentation (ESR), CA-125, and

serum CRP were elevated significantly in the chlamydia group[16]. Cytokines levels for IFN-γ, IL-1β, TNF-α, IL-6, and IL-8 were explored in a research study to investigate the role of BV in affecting serum cytokine concentration. The IFN-γ and IL-1β were increased significantly in women with BV.[6] The rising level of cytokines in both previous studies was implicated in causing preterm delivery or pelvic inflammatory disease. BV was also strongly associated with adverse pregnancy issues in a study, conducted in Saudi Arabia, that included 217 women, of which 44 women were BV positive cases.[17]

It is well accepted that the concentration of cytokines could be used as an indicator for several health issues. For instance, IL-1, IL-6, and TNF-α were measured in the peritoneal fluid to investigate their role in pelvic adhesion formation. Peritoneal fluid collection was done during laparoscopy.[18] Serum IL-6 concentration was elevated in women with endometriosis, and it was considered a reliable marker for women at risk of having endometriosis.[10] In addition, the elevated level of CRP was detected in women with polycystic ovary syndrome[19] and it is also proposed to be a serum marker in cardiovascular disease.[9] In a study conducted in Spain, which included 356 women, the high serum levels of IL-6 and reduction in vitamin D were suggested as an indicator for tubal factor infertility.[10]

In Iraq, there is little research to focus on the correlation between non-vaginosis bacteria or aerobic vaginitis and levels of cytokines, despite the much research on pathogenicity of these bacteria such as *S. aureus*, *E. coli*, and *Streptococcus spp.* which were the most frequent types of bacteria in Iraq and worldwide.[20-22] *E. coli* can have negative effects on the male and female reproductive system because it can be a part of the total ratio of etiological agents responsible for female or male infertility. In addition to its proposed role on miscarriage or stillbirth.[4] *Staphylococcus* infection can trigger a strong immune response locally or systematically. The produced cytokines influence the pituitary gland and reproductive system. The total events may lead to irregular ovulation, menoxenia, and infertility[5].

The correlation between IL-6, CRP, and vitamin D was investigated in the current research, however, the analysis did not show significant differences. CRP was weakly associated with vitamin D, and weakly inversely correlated with IL-6, but these did not reach statistical significance, probably because of the small sample size used. Several other studies showed different findings. Vitamin D was correlated inversely and significantly with CRP and IL-6 in a study that involved older subjects and their sera concentration of vitamin D.[23] In another study, vitamin D level was negatively correlated to CRP and IL-6 in acute stroke patients.[24] In a study of type 2 diabetes subjects, vitamin D supplement significantly reduced circulating CRP, but it had no significant influence on IL-6.[25] However, a review to evaluate the impact of vitamin D supplement on inflammatory markers, reported evidence to support the concept that vitamin D supplement has no or little effect on serum CRP concentration but significantly increased the concentration of IL-6.[26]

Sexually transmitted pathogens are implicated in causing infertility. *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are ranked the first in this context as major contributors to pelvic inflammatory disease or tubal factor infertility. However, limited evidence is available to discuss the impact of other vaginal microbiomes in the pathology of infertility.[27] The detection of cytokine concentration in blood sera is affordable and more accessible to any investigation. Therefore, it can contribute to determining the risk of increasing levels of some cytokines, which may refer to a potential connection between rising these levels and various health problems.

This study was conducted with the aim of investigating the levels of inflammatory markers in sera of patients with aerobic vaginitis. It can be concluded that aerobic bacteria can affect the levels of inflammatory markers (CRP, IL-6) and these markers could be risk markers for multiple diseases. Based on our data and finding of previous literature it could be concluded that a high level of CRP is an expected outcome of aerobic vaginitis. However, the rising in IL-6 level may occur when the infection develops to cause adverse reproductive system issues such

as pelvic adhesion formation, endometriosis, polycystic ovary syndrome and tubal factor infertility, which were found to be strongly associated with high levels of IL-6.

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Author Contributions

SFH conceived of the presented idea. SFH, IHM, TMM and ZFA performed the experiments. SFH and TMM helped to supervise the project. IHM and ZFA contributed to sample preparation. All authors discussed the results and contributed to the final manuscript. SFH and TMM wrote the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The protocol of the study was reviewed and approved by the Council of College of Science, University of Kerbala (Approval No. 6-1793 in 12.11.2020).

Informed consent

A signed consent form was obtained from each study participant.

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