Estimation of IL-23 Among Patients with Vaginitis and Urinary Tract Infections

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Abstract

Background: E. faecalis must first avoid being eliminated by the host's clearance mechanisms. PMNs, or polymorph nuclear leukocytes, play an important role in the immune response of humans against bacterial infections.

Objective: Estimation of IL-23 among patients with vaginitis and urinary tract infections

Materials and methods: The study's 200 participants all provided urine and vaginal specimens from patients at Baghdad's Al-Karama Hospital and Medical City Hospital over the course of three months (May to July 2022), for (18-24) hours, the materials were grown in various media. The samples were then incubated at 37 degrees Celsius for 18 to 24 hours on various selective media. Colony morphology, microscopic inspections, and biochemical assays were used by scientists first to try and identify E. faecalis. Blood sample was collected from all 200 infected patients and 44 healthy patients as control. 5ml of fresh venous blood samples were putted in gel containing tubes was allowed to clot at room temperature for 30 minutes and then centrifuged at 3000×g for approximately 3 minutes.

Results: There were positive cultures obtained from all 200 clinical samples; however, only 44 (22%) of the isolates were linked to E. faecalis. The automated Vitek 2 system used GP-ID cards with 64 biochemical assays to confirm that the isolates were indeed E. faecalis. Fast identification of 44 distinct bacterial isolates was achieved using this method, with high confidence (probability percentages of (94 to 99.7%). In this study, determination of IL-23 in E. faecalis were investigated, and the results showed that, the highest mean of IL-23 level was found among patients with UTI infected with E. faecalis (287.54±12.18ng/ml) comparing with the control group (188.08±2.29ng/ml) with a significant difference between the two groups ($P<0.05$).

Conclusions: Identification of E. faecalis by using automated Vitek 2 system more specific than other biochemical tests. Interleukin-23 is elevated in E. faecalis infection.

Keywords: *E. faecalis, UTIs, IL-23, Immunity.*

INTRODUCTION

In order to induce infection, E. faecalis must first avoid being eliminated by the host's clearance mechanisms. PMNs, or polymorph nuclear leukocytes, play an important role in the immune response of humans against bacterial infections [1]. Invading germs may be engulfed and destroyed by PMNs if they are first coated with complement proteins or particular antibodies. Opsonization is the process of covering bacteria with complement

proteins or antibodies to improve phagocytosis [2].

When studying the phagocytic death of enterococci, researchers found that PMN mediated killing was reliant on complement activation through either the classical or alternative route, rather than antibodies [3]. Antibodies against E. faecalis improved PMN-mediated death, but they were not necessary, since many trials demonstrated effective killing even in the absence of gamma globulins in the serum [4].

In order to colonize and remain inside a host, pathogens must be able to tolerate, alter, or escape immune-mediated clearance processes. E. faecalis may avoid being eaten by the host's immune cells by forming biofilms, and it can hide out within macrophages and neutrophils for long periods of time [5]. Cells in mammals use pattern recognition receptors (PRRs) to identify PAMPs, which then activate nuclear factor kappa B (NF-B)-dependent host defenses. Some of the genes regulated by NF-B include those encoding cytokines and chemokines, which are involved in the recruitment and activation of immune cells in response to infection [6].

The infection of macrophages with E. faecalis at a low multiplicity of infection (MOI = 10) activates mitogen-activated protein kinases (MAPKs) and NF-B, which in turn causes the production of pro-inflammatory cytokines [7]. Some E. faecalis strains isolated from the intestines of healthy human neonates, however, are able to inhibit MAPK and NF-B signaling and interleukin-8 (IL-8) production in intestinal epithelial cells in vitro [8].

The cellular response to E. faecalis infection in urinary tract infection (UTI) is mostly monocytic and does not need TLR2. In the bladder, neutrophils and monocyte-derived cells are activated into a robust pro-inflammatory response just by the presence of a urinary catheter [9]. Despite the robust inflammatory response elicited by catheterization, E. faecalis infection of catheterized bladders leads to the establishment of high-titer catheter-associated biofilms and bladder infection [10]. IL-23, or interleukin-23, is a cytokine whose discovery has far-reaching consequences for our understanding of chronic inflammation and autoimmune. The cytokine IL-23 is a member of the IL-12 family, which is itself a subset of the IL-6 superfamily. To present, four sequence-identical heterodimeric cytokines have been identified as members of the IL-12 family [11]. The interleukin (IL-23) protein is similar to the interleukin (IL)-6 and granulocyte colony-stimulating factor (G-CSF). To form the IL-23 receptor (IL-23R), IL-12R 1 is fused with a separate chain, IL-23R that is structurally similar to IL-6gp130. A major function for STAT3 is shared both IL-12 and IL-23's signaling pathways [12].

Antigen presentation by DCs is stimulated by IL-23, and Th17 cells are differentiated from T cells and interferon-gama (IFN-Ɣ) is produced. By directly affecting macrophages, IL-23 also functions as a terminal effector cytokine. That's consistent with an IL-23 autocrine loop acting on macrophages, at least in part. Also, peritoneal macrophages express mRNA for IL-1 and IFN-Ɣ after receiving intraperitoneal dose of recombinant IL-23 [13]. A key function for IL-23 in autoimmune illness was first indicated by studies of central nervous system autoimmunity. Both the central nervous system and inflammatory macrophages contained IL-23. Consequently, it was shown that myeloid cells in addition to T cells express their receptor, IL-23R [14].

Interleukin-23 merits close study because of its crucial function in chronic inflammation and its

efficient inhibition. To accurately interpret findings obtained employing inhibitors of varying specificities, it is crucial to keep in mind the method in which IL-23 interacts with cells (including its heterodimeric structure). To treat chronic inflammatory joint disease, IL-23 is one of the most promising targets [15]. Chronic and acute inflammatory responses are induced by pro-inflammatory cytokines such IL-1, IL-18, IL-17, IL-6, and IFN-Ɣ, all of which have a role in autoimmunity by acting on the innate or cognate stages of the process. All of these cytokines are now being studied as possible targets for autoimmune disorders [16].

Aim of study

Estimation of IL-23 among patients with vaginitis and urinary tract infections

Materials and methods

Patients admitted and seen at Baghdad's Al-Karama Hospital and Medical City Hospital throughout a three-month period (May to July 2022) provided the study's 200 participants with urine and vaginal specimens.

Ethical Approval

Getting the required ethical approval from the hospital's ethical review board, patients, and their supporters is mandatory. Additionally, prior to the collection of samples No. BMS/ 0226/016, all participants included in this work are verbally informed, and the necessary agreement for completing the tests and publishing this work is obtained from each individual.

Clinical specimens

In this article, we will go over how to properly collect samples for a bacteriological study. Those samples were carefully collected to prevent contamination.

Urine samples

Typically, the samples were from people who had UTIs. Urine samples were taken midstream and placed in sterile screw-cap containers before being inoculated on selective media (Chromo agar) and incubated aerobically at 37°C for 24 hours [17].

Vaginal swabs

Women (both pregnant and non-pregnant) with vaginitis made up the majority of the sample population. Before being removed, the swabs were put into the posterior fornix, at the top of the vagina, and rotated. The cervix was easily seen through the vaginal speculum, and swabs were massaged into and around the cervix's introits before being removed without touching the vaginal wall. The swab should be kept moist in standard saline-filled tubes until it can be transported to the lab for culture. For 24 hours, the swab was incubated aerobically at 37 degrees Celsius after being inoculated onto selective media (Chromo agar) [18].

Blood samples

Blood sample was collected from all 200 infected patients and 44 healthy patients as control. 5ml of fresh venous blood samples were putted in gel containing tubes was allowed to clot at room temperature for 30 minutes and then centrifuged at 3000×g for approximately 3 minutes. Then the sera were obtained and stored at (-20˚C) until analyses. All samples were transferred by means of a cooled box to the Faculty of Medicine Laboratory/Babylon University for the purpose of identifying the bacteria and performing laboratory analyzes.

Identification of Enterococcus faecalis with Vitek2 System

The automatic identification (ID) equipment device used in medical microbiology is a Vitek 2.

Human IL-23 ELISA kit

This ELISA kit was created using the Sandwich - ELISA principle. Each kit comes with a micro ELISA plate that has been pre-coated with an antibody specific to Human IL-23 (Interleukin-23). Samples (or standards) are mixed with the appropriate antibody and added to wells on a micro ELISA plate to get a positive result. After adding a biotinylated antibody against Human IL-23 to each microplate well, the presence of the antigen is detected using Avidin-Horseradish Peroxidase (HRP). In order to get rid of the extraneous parts, a good old-fashioned rinse is used. Each well is injected with the substrate solution. All of the wells will have a clear color except for the ones that contain Human IL-23, the biotinylated detection antibody, and the Avidin - HRP conjugate. After adding the stop solution, the blue color fades and the yellow hue remains, indicating that the enzyme-substrate reaction has been stopped. Optical density (OD) at 450 + 2 nm is estimated using spectrophotometric techniques. Human interleukin (IL-23) concentration is directly correlated with the OD value. Comparing the samples' optical densities (ODs) to the standard curve allows you to determine the concentration of Human IL-23 in the samples.

Results

The study's 200 participants all provided urine and vaginal specimens from patients at Baghdad's Al-Karama Hospital and Medical City Hospital over the course of three months (May to July 2022). The samples were grown at (37oC) for (18-24 hours) in a number of different media. The samples were then incubated at 37 degrees Celsius for 18 to 24 hours on various selective media (Chromo Agar Medium). Colony morphology, microscopic inspections, and biochemical assays were used by scientists first to try and identify E. faecalis. Only 44(22%) of the isolates were linked to E. faecalis, despite the fact that all 200 clinical samples cultured positive. To verify that the isolates were indeed E. faecalis, the automated Vitek 2 system used GP-ID cards containing 64 biochemical assays, as shown in Figure (1). Fast identification of 44 distinct bacterial isolates was achieved using this method, with high confidence (probability percentages of (94 to 99.7%). Table 1 displays the outcomes.

Table (1) Procedures for the manual and automated isolation and identification of E. faecalis using selective medium, biochemical testing, and the Vitek 2 system

No. of samples	specific media		biochemical test		automated Vitek 2 system	
	positive results	Negative results	positive results	Negative results	positive results	Negative results
200	44(22%)	-	44(22%)	-	44(22%)	-

Figure (1): Vitek 2 automated system for detecting Enterococcus faecalis

Patient Name: medical, noor
Location:
Lab ID: 183202111

Patient ID: 183202111
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Enterococcus faecalis

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	2.85 hours	**Status:**	Final
Selected Organism	99% Probability	**Enterococcus faecalis**		
	Bionumber:	156012765773471		
ID Analysis Messages				

Biochemical Details

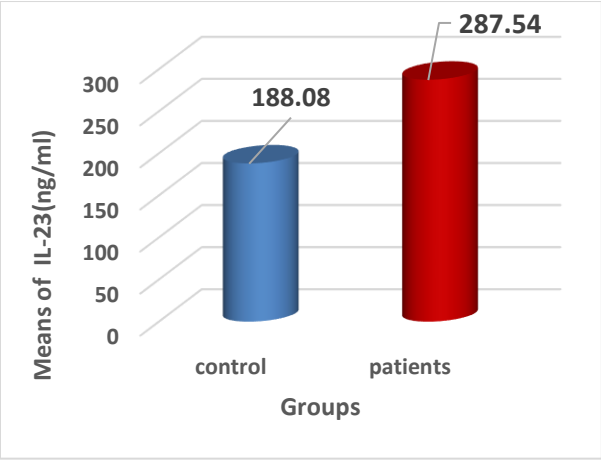
2	AMY	+	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	+
13	APPA	-	14	CDEX	+	15	AspA	+	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	+	29	TyrA	+	30	dSOR	+	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	+	39	lLATk	-	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	+
64	OPTO	+															

In this study, determination of IL-23 in E. faecalis were investigated, and the results showed that, the highest mean of IL-23 level was found among patients with UTI infected with E. faecalis (287.54±12.18ng/ml) comparing with the control group (188.08±2.29ng/ml) with a significant difference between the two groups (P<0.05), as shown in Table (2), Figure (2).

Table (2): Determination of IL-23 in E. faecalis

Parameter	sample	N	Mean ± S.E	P. value
IL23 (pg/ml)	Patients	44	287.54±12.18	0.0001
	Control	44	188.08±2.29	

Figure (2): Determination of IL-23 in E. faecalis



Discussion

These results corroborated the findings of Hashem et al., [19], who found that of 58 E. faecalis isolates from clinical specimens, 45 grew on chosen Enterococcus medium, with the majority of isolates being found in urine samples. After isolating E. faecalis from various infection sites, most frequently UTIs, Stępień-Pyśniak et al., [20] demonstrated that it is a harmful bacterium. Similar to what was found by Jahansepas et al. [21], the majority of E. faecalis isolates were found in urine samples (22.6%). Similarly, Wójkowska-Mach et al., [22] found a prevalence of E. faecalis of about 21.1% in

urine and 30% from vaginal swabs, therefore our findings are in line with theirs.

Recent years have seen a rise in interest in enterococci because of their increasing resistance to many antimicrobial medicines and their ability to cause life-threatening infections [23]. Both in and out of hospitals, urinary tract infections caused by enterococci are the most common type of sickness caused by these bacteria [24].

Patients with indwelling devices or sutured surgical wound infections were likely exposed to pathogens from the outside while in the hospital, such as bacteria that had proliferated in the patient's digestive tract and became closely associated with the patient [25]. Toc et al., [26] confirmed the species membership of 50 isolates of Enterococcus faecalis; 32% were found in feces, 32% in urine, 12% in wounds, and 10% in the vaginal canal.

Seventy-five percent of E. faecalis isolates, according to Ahmed and Hafidh, [27]. The frequency with which E. faecalis was isolated from patients was found to vary based on a number of factors, such as the sample size, the location of the study, the isolation and identification methods used, the impact of environmental conditions on patients' health, and the patients' own social and cultural background. In an antibiotic protection assay, these bacterial isolates showed competence to infiltrate a bladder cell line, and their close association with patient-derived epithelial cells was suggestive of intracellular colonization [28]. Weiner et al., [29] analyzed feces, urine, and genital fluids to identify E. faecalis.

E. faecalis has been related to the majority of instances of bacterial vaginitis (pH>4.0; E. faecalis frequently resides asymptomatically in the gut). However, if the infection spreads to other parts of the body, it might become extremely harmful. After surgery, these pathogens might enter the body through the incision site, the bloodstream, or the urinary tract. The potential for more serious infections increases when an outbreak spreads to new places. The most common cause of infectious vaginitis is E. faecalis, which causes a white, watery discharge, a fishy odor, and an elevated vaginal pH [30].

Wang et al., [31] discovered that, IL-23 regulated these pathways in human macrophages in a similar fashion. These findings underline the critical role for IL-23 in mediating antimicrobial actions in macrophages, which may help explain why pharmacological inhibition of the IL-23 pathways increases infection susceptibility. And they point out that even people who are protected from other immune-mediated disorders by carrying the IL-23R gene may be vulnerable to bacterial infection.

To explain the inflammatory response mechanism generated by E. faecalis, Bloemendaal et al., [32] observed that infection with this bacteria causes the release of IL-23. In addition, E. faecalis was shown to activate caspase-1 and cause IL-23 production by Antushevich, [33].

Multiple prior investigations have used immunohistochemical staining and enzyme-linked immunosorbent assay to detect elevated IL-23 levels in patients with E. faecalis infection [33]. It is crucial that the host's adaptive and innate immune systems are able to distinguish between pathogenic organisms and the commensal flora in order to maintain mucosal homeostasis [34].

Conclusion

Identification of E. faecalis by using automated Vitek 2 system more specific than other

biochemical tests.Interleukin-23 is elevated in E. faecalis infection

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