

Immunoglobulin –E (IgE) levels in patients with wounds infection caused by different bacterial species

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Abstract

This study is designed to determine the immunoglobulin-E (IgE) levels in patients with wound infections caused by different bacterial species. Fifty five samples were collected from patients suffering from wounds, including pus swabs and blood samples for both sexes, who attended Al-Salam teaching Hospital and some outpatient clinics in Mosul City, with patients aged (8-50) years. During the time period of September 2022 to the end of March 2023. The data is entered into a questionnaire for the individuals that are the study's subject. To perform required laboratory tests, samples were sent to the lab. That detected (7.27%) include 4 patients were negative growth, and (92.72%) include 51 patients were positive growth. (87.27%) of samples had only one species isolated from each sample, while (12.72%) include 7 patients were mixed cultures isolated from the total swabs. *Staphylococcus aureus* and *Escherichia coli* represented the highest rates among bacterial isolates at (33.33%) include 18 patients and (20.37%) include 11 patients respectively; *pseudomonas aeruginosa* and *staphylococcus epidermidis* were at (14.81%) include 8 patients, while a lower percentage of (10%) include 5 patients was for *Klebsiella pneumoniae*. Antibiotic-resistant bacteria included isolates of *Staphylococcus aureus*, *E. coli*, *Staphylococcus epidermidis*, *pseudomonas aeruginosa*, and *K. pneumoniae*. Antibiotics included (Clindamycin (10 g/disc), Cefixime (5g/disc), Imipenem (10 g/disc), and Amoxicillin/Clavulanic acid (20/10 µg/disc). The largest percentage of gram-positive and gram-negative bacteria was in the age groups (21-30) years then (41-50) years, while the lowest percentage rate was in the age group (1-10) years. IgE showed the highest level ($P \leq 0.05$) reached at mean \pm S.D (0.315 \pm 0.023ng/ml) with *Pseudomonas aeruginosa* infection and the lowest level ($P \leq 0.135$) at (0.141 \pm 0.072) with *Staphylococcus epidermidis*, compared with the control group where mean \pm S.D was (0.178 \pm 0.056).

Introduction

The skin is one of the important organs that covers the human body, as well as protecting it from external influences. Therefore, Human skin serves as one of the initial lines of defence against pathogens and serves as the body's primary barrier. Due to its physical characteristics, such as its nearly total resistance to moisture and ability to stop the infiltration of liquids that

would otherwise immerse human tissues, the skin serves to protect the body. However, microbes may be able to enter the body through inconspicuous cutaneous breaks. [1].

Most specific antibiotic resistant bacterial strains are composed of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* [2]. Over a period of an individual's life, keratinized skin cells, immune cells, and microorganisms all work together to maintain the skin's physical and immunological barrier under both homeostatic healthy settings and under various stressors, such injury or infection [3]. The cause of wounds might be accidental or purposeful, a disease process, or both. To repair tissue integrity after an injury, several cellular and extracellular pathways are activated in a carefully controlled and coordinated process [4].

There are many different reasons wounds occur, including surgery, traumas, extrinsic forces (such as pressure, burns, and cuts), and pathologic illnesses like diabetes or vascular diseases [5]. The most common bacterial pathogens associated to wound infection include *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Proteus species*, *Streptococcus species*, and *Enterococcus species* [6]. The Y-shaped proteins known as human immunoglobulins are made up of two identical light chains (LCs) and two identical heavy chains (HCs). In naturally occurring systems, one LC and one HC join up with another identical heterodimer to generate a complete immunoglobulin. [7]. The similar effect is achieved by anti-IgE therapy by inhibiting IgE from interacting with mast cells' and basophils' receptors [8].

Aim of study

To determine the relationship between immunoglobulin E and some bacterial that caused wound infections

Materials and methods

Samples collection

From the third last week of September 2022 and finished in the last week of March 2023. 55 samples, including blood and wound swabs, were taken from patients with wound infections who were admitted to AL-Salam teaching hospital, consulting clinics, and outpatient clinics. Patients were of both sexes (males and females), and ranged in age from (8 to 82) years. To perform the necessary laboratory tests, samples were sent to the lab.

Sample culturing

The sample was cultivated on a variety of media, including mannitol salt agar, blood agar, nutritional agar, MacConkey agar, and Eosin Methylene Blue agar. These plates were incubated for 18 to 24 hours at 37 °C. A microscopic analysis was then completed.

Diagnosis

Based on the appearance of the bacterial colonies' growth during incubation, and microscopic examination, the cultures were identified. To identify the bacterial species in isolates, some biochemical tests including urease, TSI, catalase, oxidase, IMVIC, and coagulation assays were carried out. [9]. For confirmation of the diagnosis, API 20 E, API STAPH and Vitec 2 compact system technique were applied.

Antibiotic sensitivity test

For each isolated bacterial strain, an antibiotic sensitivity test was performed. by the spread plate method, according to [10]. Using 5 antibiotics: Clindamycin (10µg/disc), Amoxicillin/Clavulanicacid(10µg/disc), Cefixime(5µg/disc), Imipenem(10µg/disc) and Amoxicillin/Clavulanicacid (20/10 µg/disc) made by / BIOANALYSE (Turkish company). The diameter of the inhibition zone, which is a clear zone surrounding the antibiotic discs, was used to measure the bacteria's sensitivity to these antibiotics.

Immunoglobulin-E (IgE) levels test:

For the IgE test, serum samples from the same individuals with wound infections' blood were also taken. IgE levels in the patient's serum were detected using the ELISA technique and in accordance with the instructions provided in the test kit's manufacturer's manual (Sunlong Company).

Statistical analysis

Using Microsoft Windows 7 EXCEL (version 2010) and SPSS version 23 (independent T.T. test and ANOVA for Leas Significant Difference LSD), all data from the current study were statistically analysed.

Results and Discussion

Samples distribution according to the bacterial growth

According to the results shown in Figure 1 samples from 51 samples (92.72%) showed bacterial growth (positive culture) on culture media, whereas 7.27% did not (negative culture).

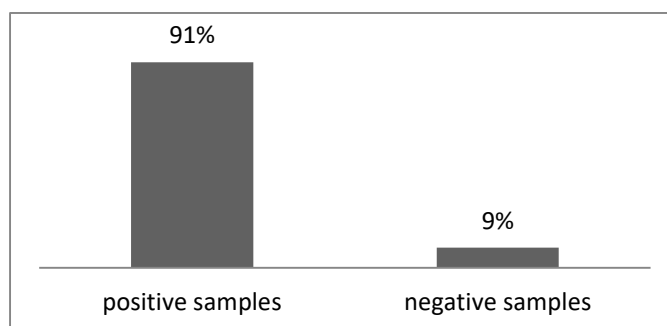


Figure 1: Distribution of samples based on bacterial growth

The study [11]. approached the current study, that found the bacterial culture on different media was (87.4%) and samples that showed a negative growth on media were (12.6%). The difference in results in this study may be due to the length of time and the number of patients included in the study.

Bacterial isolates are arranged and distributed according to age groups.:

Table 1 display the amount of bacterial isolates that were isolated from wound infections and distributed based on age groups

The ages (Years)	G+ bacteria		G-bacteria		The total (%)	Patients number
	number	%	number	%		
1-10Y	1	1.2	2	2.5	3.7%	3
11-20Y	5	6.4	4	5.1	11.5%	9
21-30Y	10	12.8	11	17.9	30.7%	24

31-40Y	4	6.4	4	5.1	11.5%	9
41-50Y	6	7.6	7	8.9	16.5%	13

In general, the maximum rate of bacterial infections was in the age group between (21-30 years) at 24 (30.7%), followed by the age category confined between (41- 50 years) at 13 (16.5%). while the category at the age group (1-10) represented the lowest rate at 3 (3.7%). The age groups (11-20 and 31-40 years) had the same percentage of 9 (11.5%). As in the distribution of bacterial isolates with age groups, the same percentage levels appeared when distributing bacterial isolates with acceptance of the Gram stain. Gram-positive bacteria had the lowest percentages (1.2%) and gram-negative bacteria had the highest percentages (2.5%) in the age group (1-10 years), whereas the remaining bacterial isolates were distributed among the age groups in a manner that was restricted to these two patient categories. Gram-positive bacterial isolates were least prevalent in the age group (12.8%), whereas Gram-negative bacterial isolates were most prevalent in the age group (21-30 years) his result agreed with the research [12]. The rate of isolation in this study was at its highest level. The age range of the group is (15 – 35) years, and the rate of isolation decreases with age. This may be because adolescents experience high levels of physical and hormonal activity, as well as continual movement, exploration, and social interaction. As a result, they may be more likely than other age groups to contract certain diseases, and do so at a higher pace [13].

Distribution of bacterial isolates type in Wound infection samples

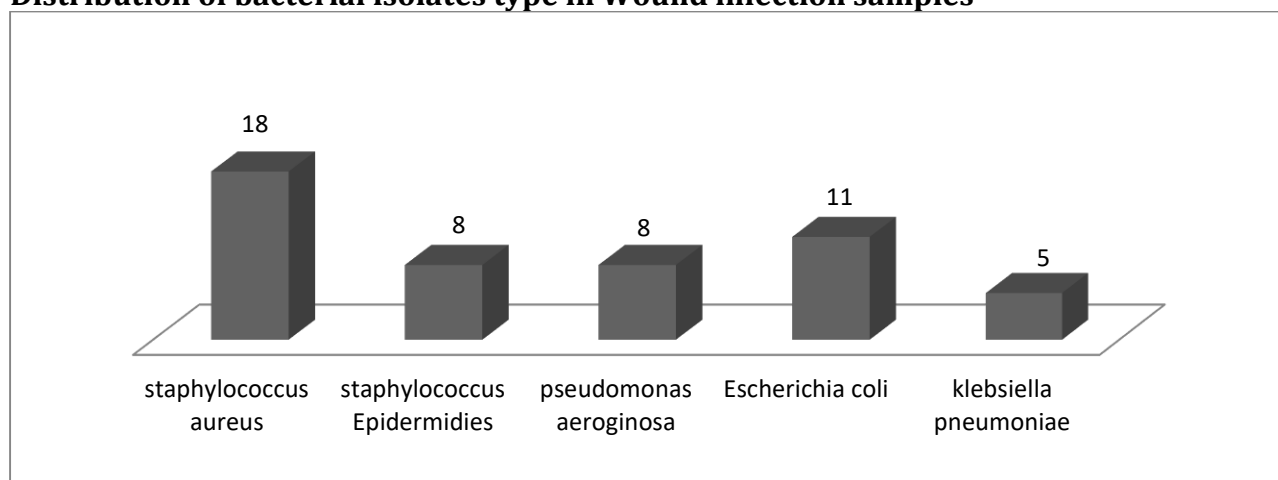


Figure 2: distribution of the different strains of bacteria in samples taken from patients who had wound infections.

50 bacterial isolates were collected from wound infections through the culture of samples, as shown in "Fig. 2," with *Staphylococcus aureus* 18 isolates having the largest proportion of gram-positive bacteria (36%), followed by *Staphylococcus epidermidis* 8 isolates with a percentage (16%)., whereas gram- negative bacteria, *E.coli* represented the highest percentage of gram-negative isolates with 11 isolates (22%), followed by *pseudomonas aeruginosa* at 8 isolates with a percentage (16%) followed by *Klebsiella pneumoniae* at 5 isolates with a percentage (10%). Due to their widespread nature, their role in nosocomial infections acquired in hospitals, and the fact that they are resistant to both standard antibiotics and hospital-use disinfectants, most of wound infections are caused by these bacteria. The results of the study agreed with [14]. *S. aureus* and *P. aeruginosa* are often found

growing in combined co-cultures in many different types of chronic wounds. [15]. The most common bacteria found were *Corynebacterium*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli*, and *Staphylococcus aureus*. *Enterococcus cloacae* was the most typical species of *Enterococcus*. [16].

determining the sensitivity of bacterial isolates to different antibiotics

Table 2: bacteria's susceptibility to several antibiotics

Bacterial type	(n.)	Clindamycin		Imipenem		Amoxicillin/Clavulanic acid		Cefixime	
		10 µg/disc		10µg/disc		20/10 (µg/disc)		5µg/disc	
		R	%	R	%	R	%	R	%
<i>Staphylococcus aureus</i>	18	1	5.5%	0	0%	4	22.22%	17	94.4
<i>Staphylococcus epidermidis</i>	8	3	37.50%	7	87.5%	4	50%	7	87.5
<i>Pseudomonas aeruginosa</i>	8	2	25%	0	0%	8	100%	7	87.5
<i>Escherichia coli</i>	11	8	72.72%	1	9.09%	5	45.45%	11	100
<i>Klebsiella pneumoniae</i>	5	2	40%	1	20%	1	20%	5	100

R= resistance

Based on the findings in Table 2, the growth of bacteria that are resistant to several antibiotics necessitates the development of new treatments to deal with this serious problem. [17]. Study showed that *staphylococcus aureus* had (22.22 %) resistance to amoxicillin and clavulanic acid was approached for study [18,19]. *Staphylococcus aureus* resistance to imipenem was 0% and very low resistance was discovered against clindamycin (5.5%); however, the resistance to amoxicillin/clavulanic acid was found to be (19.23%) (26.7%). In comparison studies [20]. were the *staphylococcus aureus* resistance to Imipenem (2%) and to Clindamycin (7%), but was relatively far to comparative study [19]. whereas the *staphylococcus aureus* resistance to Cefixime was (94.4%) in current study, the *staphylococcus aureus* showed resistance to Cefixime (100%) in the comparative study [21]. In the current study, *Staphylococcus epidermidis* resistance to amoxicillin/clavulanic acid was (62.5%), which is comparable to the comparative study [19], where resistance ratio was (77.8%), but current study result about Amoxicillin/Clavulanic acid resistance was relatively far to another comparative study which was (14.3%)., the resistance of the *staphylococcus epidermidis* to Clindamycin in current study (37.50%) approached to result in comparative study [23]. *Staphylococcus epidermidis* showed resistance to Cefixime (87.5 %), compare to the result in comparative study [33], which was (100%), whereas resistance to Cefixime in the current study showed high resistance to Cefixime according to study [24], which was (29.90%), *Staphylococcus epidermidis* showed resistance to Imipenem Which was (87.5%), this result matched with the study [25]. Resistance to Imipenem was (88.2%), whereas approached to study [26]. Which resistance to Imipenem (100%) As for *E.coli* bacteria presented (9.09 %) resistance to Imipenem of , and this agreed with result of research [27].were result was (5%) and relatively far away to result in another comparative study [28]were the resistance was (0%). *Escherichia coli* showed (45.45 %) resistance to Amoxicillin/Clavulanic acid matched with study [28], where the resistance to Amoxicillin/Clavulanic acid (50%). but not agreed in the another comparative studies [29,30], which resistance was (33.3%), (79.6%). about Cefixime *Escherichia coli* bacteria showed (100%) resistance percentage to Cefixime

that agreed with researcher [30], where (100%) resistance percentage to Cefixime. *Escherichia coli* bacteria showed resistance to Clindamycin *Klebsiella pneumonia* bacteria showed (20%) resistance percentage to Amoxicillin/Clavulanic acid, that Agreed with to study [19], showed (20%) resistance percentage Amoxicillin/Clavulanic acid, but the result in Current study not matched with another comparative study [31], *Klebsiella pneumonia* showed (47.1%) resistance to Amoxicillin/Clavulanic acid. *Klebsiella pneumonia* showed (20%) resistance to Imipenem that close to studies [32,33]. where the resistance to Imipenem was (25%), (25.4%). *Klebsiella pneumonia* showed (100%) resistance to Cefixime that matched with study [33], where the resistance to Cefixime (100%) but not agreed with study [34], where the resistance percentage (14.30%). *Klebsiella pneumonia* showed (40%) resistance percentage to Clindamycin whereas resistance percentage to Clindamycin in comparative study [33], which was (6.7%) resistance percentage to Clindamycin, *Pseudomonas aeruginosa* showed a high rate of resistance to many antibiotics, including clindamycin. where the resistance percentage was (25 %) this result matched the [36], were the result was (21.3%). *Pseudomonas aeruginosa* showed resistance to Imipenem, and Cefixime were the resistance percentage (0%), (78.5) this result agreed with the study [37]. about Imipenem resistance where the result was (0%) and approached with Cefixime where the result was (100%) and the result of current study about Imipenem resistance agreed also with result of another comparative study [38], which also was (0%). *Pseudomonas aeruginosa* showed resistance to Amoxicillin/Clavulanic acid which was (100%) resistance percentage that matched with result in comparative study [39]. which also resistance percentage to Amoxicillin/Clavulanic acid was (100%).

Immunoglobulin -E (IgE) concentration

The current study's results show significant differences. the level of $P \leq 0.01$ of Immunoglobulin concentration IgE (0.228 ± 0.713 ng/ml) among people who had wound infections compared to its level in control groups (0.174 ± 0.554 ng/ml) as shown in Table 3.

Table 3: The concentration of IgE (ng/ml) for wound infection

Type of infection	conditions	(IgE) Mean (ng/ml) \pm S.D.
Wound infections	Patients	0.228 ± 0.713
	Controls	0.174 ± 0.554
P-Value	0.008	

The relationship between bacterial isolates type and Immunoglobulin E levels

The results showed in Table 4, In patients with various bacterial types of infection as compared to the control sample, the amount of IgE content was varied.

Table 4: The relationship between bacterial isolates type and Immunoglobulin E levels

Bacterial that caused infection	Classes	Mean(ng/ml)± S.D.	P-value
<i>Pseudomonas aeruginosa</i>	patients	0.315±0.023	0.016**
	controls	0.181±0.060	
<i>E.coli</i>	patients	0.249±0.222	0.002**
	controls	0.174±0.055	
<i>Klebsiella pneumonia</i>	patients	0.146±0.961	0.296**
	controls	0.118±0.073	
<i>Staphylococcus aureus</i>	patients	0.202±0.121	0.031**
	controls	0.174±0.055	
<i>Staphylococcus epidermidies</i>	patients	0.141±0.072	0.293**
	controls	0.178±0.056	

A standard deviation (S.D.) =is a measure of how dispersed the data is in relation to the mean Mean= is the average of the given number. (ng)= nano gram. (ml)= milliliter. ** (P≤0.05).

P-value=measures the probability of obtaining the observed result, assuming that the null hypothesis is true.

The results showed in Table 4 Patients with *Pseudomonas aeruginosa*-related skin infections had the highest mean levels of IgE was at (0.315 ng/ml) matched with control (0.181 ng/ml) in individuals with *staphylococcus epidermidies* infection, and the lowest mean level of IgE was at (0.141 ng/ml) compared with control (0.178 ng/ml).

IgE, which plays a significant role in allergic and inflammatory reactions and is high in allergic illnesses, can harm tissue or organs. The high-affinity IgE receptor Fc receptor 1 (FcR1) is widely expressed in leucocytes [40]. Mast cells are found almost everywhere in tissues and are commonly found next to epithelia, fibroblasts, blood and lymphatic arteries, and neurons. They are frequently stimulated by immunoglobulin E (IgE) antigen cross-linking, which causes the cells to degranulate and release a variety of pre-formed molecules, such as histamine, serotonin, tryptase, and chymase, as well as by IgE-independent non-allergic responses, which cause the release of both pre-formed and newly synthesised MC mediators, such as cytokines and chemokines [41].

Conclusions

The study found that IgE concentration is significantly increased in those patients had wounds or surgeries and in those who had been diagnosed with bacterial growth. In case of *Pseudomonas aeruginosa* infection the highest IgE is observed and in case of *Staphylococcus epidermidies* the lowest IgE is observed, this finding highlights the of in the body's immune system.

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قياس مستويات الغلوبولين المناعي (امينوكلوبيولين ايبسلون) في المرضى الذين يعانون من التهابات الجروح والتي تسببها أنواع بكتيرية مختلفة

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الخلاصة:

اجريت الدراسة الحالية لتقدير مستوى تركيز المتغير المناعي الامينوكلوبيولين E في المرضى الذين لديهم جروح ملتهبة بواسطة انواع بكتيرية مختلفة. شملت الدراسة 55 عينة اخذت بشكل مسحات وعينات دم من مرضى المصابين باخماج الجروح المراجعين لمستشفى السلام التعليمي وبعض العيادات الخارجية في مدينة الموصل وبأعمار تراوحت بين (8-82) سنة ومن كلا الجنسين، للمدة من نهاية ايلول 2022 والى نهاية اذار 2023. وقد سجلت المعلومات الخاصة بالبحث في استمارة استبيان خاصة بمعلومات عن الشخص ضمن موضوع الدراسة. اظهرت نتائج المزارع البكتيرية ان (7.27%) شملت 4 مرضى كانت نتيجة الزرع البكتيري سالبة النمو، و (91%) شملت 51 حالة مرضية كانت ايجابية النمو، وكانت العينات بنسبة (88%) التي ظهر بها نوع بكتيري واحد فقط وبينما كانت (12%) من المزارع مختلطة النمو من المسحات الكلية احتوت على اكثر من نوع بكتيري. تمثل المكورات العنقودية الذهبية والإشريكية القولونية أعلى المعدلات بين العزلات البكتيرية إذ بلغت 18 (33.33%) و 11 (20.37%) على التوالي، أما الزائفة الزنجارية والمكورات العنقودية البشرية فقد بلغت 8 (14.81%) بينما كانت النسبة أقل من 5 (10%) للكليسيلا الرئوية. كانت العزلات البكتيرية (المكورات العنقودية الذهبية، الإشريكية القولونية، والمكورات العنقودية البشرية، الزائفة الزنجارية، والكليسيلا الرئوية)، مقاومة للمضادات الحيوية (كلندامايسين 10 ميكروغرام / قرص)، (سيفيكسيم 5 ميكروغرام / قرص)، (امبينيم 10 ميكروغرام / قرص)، (اموكسيسيلين / حمض الكلافونييك 20/10 ميكروغرام / قرص). كانت أكبر نسبة للإصابة في الفئات العمرية (21-30) سنة وتلتها الفئة العمرية (41 - 50) سنة، بينما كانت أقل نسبة مئوية في الفئة العمرية (1-10) سنوات، وأظهر الكلوبولين المناعي ايبسلون أعلى مستوى ($P \leq 0.01$) عند (0.023 ± 0.315 نانو كرام / مل) مع عدوى الزائفة الزنجارية وأقل مستوى ($P \leq 0.01$) بلغ (0.141 ± 0.072 نانو كرام / مل) مع البكتريا الكروية العنقودية الجلدية مقارنة مع الفئة الضابطة إذ كانت (0.178 نانو كرام / مل).

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