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ORIGINAL ARTICLE

Assessments the Role of Neutrophil CD64 in Patients with Sepsis in Al- najaf Al- Ashraf Province

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KEYWORDS

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ABSTRACT: Sepsis is a global health matter that provides a considerable danger of death. The main objective of this investigation was to assess the use of CD64 and IgG in the development of bacterial sepsis in patients infected with (Salmonella typhi and Klebsiella pneumonia), Gram-positive bacteria Staphylococcus aureus and the correlation of the marker (CD64) with bacterial sepsis. This study was carried out with a total (140) individual of both sex (100) suspected sepsis patients and (40) healthy group with age ranged (13-65) year enrolled in this study. The result of Microbiological tests was found 40 specimens contain bacterially isolated, was the frequency among 30 (75%) male and 10(25%) female and result revealed that 10 (25%) specimens as a Gram-positive isolate (S. aureus) and 30(75%) specimens as Gram-negative bacteria (S. typhi, K. pneumoniae) while 60 of the rest specimens did not show any growth. While the current study, 30 Gram-negative isolates appeared as a positive result for K. pneumoniae (6) and (24) for S. typhi isolates and represented a major cause for sepsis by using the VITEK system to confirm all bacterial isolates. This study concluded that the sepsis disease influences some risk factor such as age, sex, place of living and the type of bacteria, also affected on immune response represented by CD64.

INTRODUCTION

Sepsis remains a significant public health challenge common because of prolonged inflammation, immune suppression, awareness of diseases, and yet death [1]. It implies a life-threatening situation affected by the body's response to an infection. The body usually issues chemicals within the bloodstream to challenge the infection. Sepsis happens if the body's excessive reply to these chemicals, triggering switches and diseases that can destroy multiple organ rules [2].

Bacteremia created by *K. pneumoniae* does see more, including a poorer diagnosis, into patients with underlying diseases because of potential immune system deterioration [3]. Hence, controlling subordinate diseases remains highly relevant for decreasing the death due to sepsis-induced by *K. pneumoniae*.

Salmonella enterica serovar *Typhi (Salmonella Typhi)* creates typhoid fever (TF), a disease that is popular in regions with inadequate sanitation and lack path to safe food and water [4].

Flow cytometric (FCM) estimate of neutrophil CD64 (nCD64) expression assay and also monocyte human leukocyte antigen-DR (mHLA-DR) assay has achieved high sensitivity and specificity [5].

CD64 remains one of the immunoglobulin receptors exposed at monocytes and eosinophils. CD64 expression does below on resting neutrophils, and it is rapidly deregulated following activation with bacteria. If the bacteria are removed, the expression of neutrophil CD64 (nCD64) will dramatically reduce in 48 hours and be back to normal levels within seven days. As a result, nCD64 is an important potential marker in diagnosing bacterial infections and sepsis; IgG's high-affinity receptor is CD64. Inflammatory cytokines on neutrophils have little effect on it. In SIRS or sepsis, CD64 overexpression is connected to PMN activation [6]. This study aimed to investigation of common bacteria among patients with sepsis and the assessment of immune response by CD64.

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MATERIALS AND METHODS

Blood samples were collected, which included 100 suspected septicemia samples, during the period from the beginning of November 2020 until the end of January 2021, suspected patients, the proportion of male and female patients and determining the location of the patient's living location. The blood was drawn directly from the patient to measure the immunological parameters, which are measured by flow cytometry. First, the patients were interviewed directly using an anonymous questionnaire that included patient details and histories. This study was consistent with the ethics of the specialist center and verbal informed consent was obtained from all participants.

The control samples were equal with the patient samples in terms of number, age ratio, sex ratio of males and females, and the place of living also countryside and city. Also, ask the question to the control whether he had taken a treatment close to the patient's samples and that by making a special question sheet for the control samples. Where blood was drawn from a vein to measure immunological parameters, also for the flow cytometry.

Initial diagnosis, and detection of the presence of bacteria in the blood, is suspected according to the following source [7].

After the diagnosis and confirmation of the presence of bacteria in the blood by bact alert 3D system, withdraw 3 ml a second time from the patient, and the blood samples were divided into two parts. The first part (1.5 ml) was transferred to an anti-coagulant tube from the two study groups and stored for a period of no more than 48 hours to be used in the CD64 immunoassay with a flow cytometry.

Smears of all isolates were stained using the Gram's process and examined under the oil immersion lens of a light microscope at a magnification of X 100 to determine the staining effect, cell size, shape, and design [8].

All *S. typhi* strains and *S. aurues* strains and *K. Pneumoniae* strains were identified by using Vitek2® system (BioMerieux® -France) according to steps of Manufacture Company. The following procedure was used to estimate the CD64 by flow cytometry technique 50 µl of anticoagulated (EDTA, ACD) whole blood was added to the bottom of a 12-75 mm polystyrene tube, along with the conjugated antibodies, as directed by the manufactures product insert. Vortex and incubate in the dark, at room temperature for the time specified, then 100 µl of reagent A was added to each sample and work it vortex. Incubate for 10 minutes at room temperature in the dark, after that 1ml of reagent B was added to each sample and work it vortex. Incubate for 20 minutes in the dark, finally analyze the sample on the flow cytometry or store at 2-8 C in the dark room until analysis and estimation the results by used standard curve is only for demonstration purposes, code number for CD64 kit A100.

RESULTS

The result of Microbiological tests was found 40 specimen contain bacterial isolated, was the frequency among 30(75%) male and 10(25%) female and result revealed that 10 (25%) specimens as a Gram positive isolates (*S .aureus*) and 30(75%) specimens as Gram negative bacteria (*S .typhi , K .pneumoniae*) while 60 of the rest specimens wasn't show any growth.

So that the present study conducted that the number of patients with sepsis according to the type of Gram stain separated to Gram-negative bacteria (*S. typhi* and *K. pneumonia*), Gram-positive bacteria *S. aureus* as shown in Table 1.

Table 1. The percentages of bacteriologic growth.

Bacterial growth	Isolates numbers	Pecentages
Gram negative bacteria	30	75%
Gram positive bacteria	10	25%
No growth	60	60%

There were 40 sepsis patients and 40 apparently healthy individuals enrolled in this study. Both groups were matched for their demographic characteristics; age, sex, Occupation and Residence, in all comparisons, P. value > 0.05, not significant Furthermore, in both groups, males were dominant contributed for 75% as seen in Table 2.

The 40 samples that contained bacterial growth were divided according to sex into 30 (75%) male and 10 (25%) female as shown in Table 2. And it was divided according to the occupation of the sepsis patient into the unemployed 26 (65%) patients, the workers 4 (10%) and the 10 (25%) students. The sepsis samples were divided according to the place of residence of the patient to the city population 22 (55%) patient and to the countryside 18 (45%) patient it is presented in Table 2.

Table 2. Demographic of study population

Variable		Groups				P. value*
		Sepsis patients		Controls		
		No.	%	No.	%	
Age (year)	< 20	8	20.0%	7	17.5%	0.992
	20 – 29	4	10.0%	5	12.5%	
	30 – 39	14	35.0%	15	37.5%	
	40 – 49	10	25.0%	9	22.5%	
	≥ 50	4	10.0%	4	10.0%	
Sex	Male	30	75.0%	30	75.0%	1.00
	Female	10	25.0%	10	25.0%	
Occupation	Unemployed	26	65.0%	26	65.0%	1.00
	Employed	4	10.0%	4	10.0%	
	Students	10	25.0%	10	25.0%	
Residence	Urban	22	55.0%	22	55.0%	1.00
	Rural	18	45.0%	18	45.0%	

*both groups were matched for baseline characteristics , P. value not significant > 0.05

And the distribution of sepsis patients according to age from 13 to 65 years old, and the most vulnerable age group was between (30-39) years, where the percentage was 35%, because of the higher numbers of bacteria in this age group about 14 bacteria, while the lowest age group exposed to infection was between 20 and 29 years and the percentage was 10% because of the low numbers of bacteria in this age group about 4 bacteria, and also the age of over 50 years old to be the least susceptible group, also 10%, and also because of the low numbers of bacteria about 4 in this age group according Table 2.

In the current study, from 40 specimens that found as Gram negative bacteria, 30(75%) isolations and Gram positive bacteria, 10(25%) isolations have been appeared as positive results for *K. pneumoniae* their number appeared about 6 and *S. aureus* about 10 and *S. typhi* 24 isolates and represented a major cause for sepsis. (Figure 1).

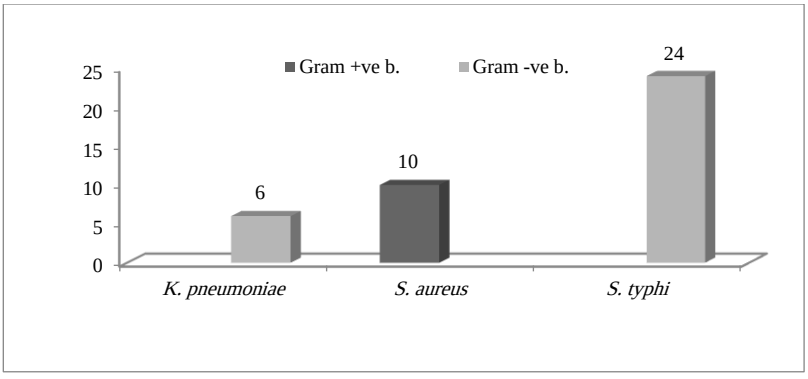

Figure 1. Number of patients with sepsis according to the type of Gram stain. N= 40 patients.

Finally, the last confirmatory step for detecting *S. typhi*, *S. aureus* and *K. pneumoniae* after biochemical tests and finally diagnosed under study was done by using VITEK system and according Table 3 revealed the numbers diagnostic bacteria where the number of *S. typhi* bacteria was about 24 (60%), *S. aureus* 10 (25%) and *K. pneumonia* about 6 (15%) . The accuracy of the VITEK 2 system for direct identification and susceptibility testing in Gram-positive rods and Gram-negative cocci blood cultures varied depending on the type and species of bacteria in a prior study.

Table 3. The numbers of bacteria identified by the VITEC device for sepsis patients

Bacterial SSP.	No.	Percentage
S. typhi	24	60%
K. pneumoniae	6	15%
S. aureus	10	25%

To assess the possible relationship and confounding effect of demographic variables on the frequency distribution sepsis according of types of bacteria and possible effect on the markers levels, two analyses were performed; first using cross-tabulation between demographic variables from one side against types of bacteria on the other side, this analysis revealed no significant association with all variables, in all comparisons, P. value > 0.05, not significant, Table 4.

Table 4. Relationship between type of bacteria and demographic characteristics of sepsis patients (N = 40)

		Type of bacteria			Statistical test*	P.value
		S. typhi	***S. aureus***	***K. pneumoniae***		
		No.	**No.**	**No.**		
Age (year)	< 20	5	1	2	7.11	0.497 ns
	20 – 29	3	1	0		
	30 – 39	5	6	3		
	40 – 49	7	2	1		
	≥ 50	4	0	0		
Sex	Male	16	8	6	2.52	0.267 ns
	Female	8	2	0		
Occupation	Unemployed	15	7	4	1.057	1.00 ns
	Employed	3	1	0		
	Students	6	2	2		
Residence	Urban	11	7	4	1.91	0.407 ns
	Rural	13	3	2		
Pathological conditions	Pneumonia	7	3	0	14.57	0.249 ns
	Diabetic foot /leg amputation	6	0	2		
	Renal failure on dialysis	1	3	2		
	Unconscious ICU patient	2	1	1		
	Heart surgery	3	1	0		
	Endocarditis	3	0	1		
	Respiratory failure	1	1	0		
	Covid-19/Heart failure	1	1	0		

*Fisher's exact test used in all comparison

While the current result revealed the number of patients with sepsis according to the type of bacteria were total 24 patients infected with *S. typhi* separated to 16 male and 8 female also revealed 6 patients male infected with *K. pneumonia* bacteria as well as 10 patients infected with *S. aureus* isolated to 8 male and 2 female as shown in Figure 2.

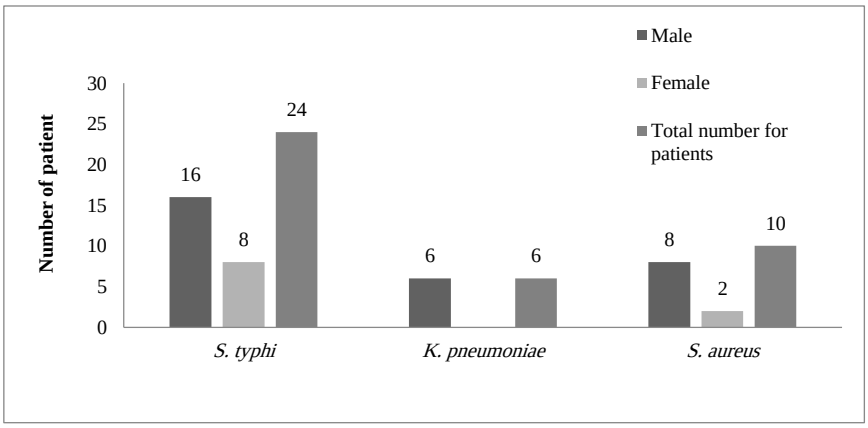

Figure 2. Number of patients with sepsis according to the type of bacteria. N= 40 patients.

As a result, the current research attempted to determine the immunological and bacteriological characteristics of sepsis patients using CD64marker was measured by the flow cytometry technique. the total of 40 sepsis patients and 40 healthy controls were enrolled in this study, and both groups were matched for baseline characteristics; this matching was done to control the confounding impact of these variables on the study's outcome, As show in Table 5 and Figure 3 the results revealed the mean CD64 concentration level the of sepsis patients group was higher than the control group with highly significant difference; the mean CD64 was 65 ± 14.2 vs. 25.9 ± 3.3, respectively, (P. value < 0.001).

Table 5. Comparison of CD64 marker level in Sepsis patients and control groups (N = 40).

Marker	Statistics	Groups		Statistical Test	P. value
		Sepsis patients (n = 40)	Controls (n = 40)		
CD64 (ng ml^{-1})	Mean ± SD	65.0 ± 14.2	25.9 ± 3.3	*t* = 11.5	**< 0.001**
	Minimum	25.2	20.0		
	Maximum	90.1	31.0		

SD: Standard Deviation of mean; t : Student's t test for two independent samples

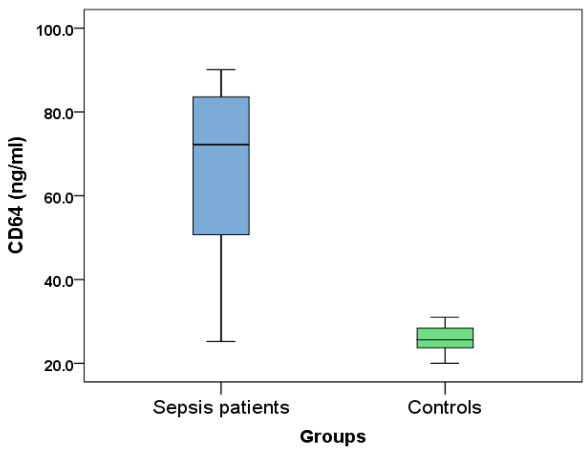

Figure 3. Comparison of CD64 in both studied groups.

CD64 level in this study was measured by using flow cytometry technique code number A100, Comparison of mean CD64 across the type of bacteria, revealed that sepsis patients with *S. aureus* had the higher CD64 level (86.78 ng ml^{-1}) compared to those with *S. typhi* (63.69 ng ml^{-1}) and those with *K. pneumonia* (33.65 ng ml^{-1}) which was the lowest level, (P. value < 0.001), Table 6 and Figure 4.

Table 6. Comparison of CD64 levels according to type of bacteria in sepsis patients (N = 40)

Type of bacteria	CD64 (ng ml^{-1})		Statistical test	P. value
	Mean	SD		
S. typhi	63.69	16.21	ANOVA F = 28.9	< 0.001 sig
S. aureus	86.78	2.81		
K. pneumoniae	33.65	12.03		

SD; Standard Deviation of mean, sig: significant.

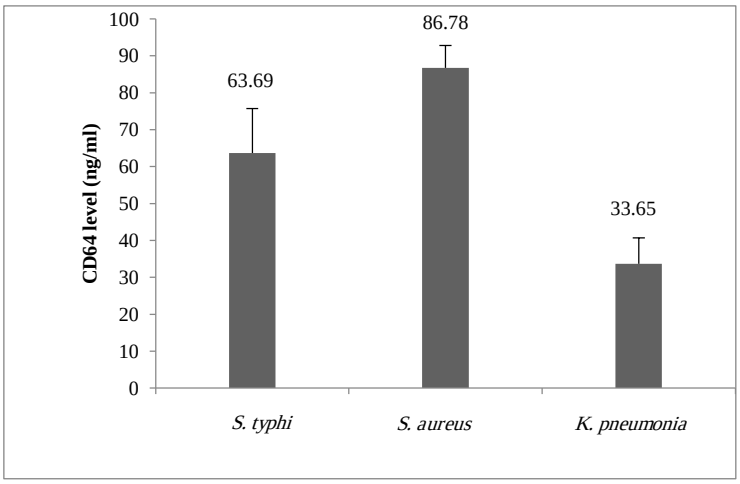

Figure 4 . Comparison of mean CD64 level according to type of bacteria in sepsis patients (N = 40).

To assess the validity of CD64 as predictor of sepsis, Receiver operating characteristics (ROC) curve investigation was conducted for each test individually. It is with mentioned that ROC curve plot the true positive rate of a test vs. its false positive rate, giving an area under the curve (AUC) as an indicator for validity of a test, the higher AUC close to one the more valid test. For CD64 , the AUC was 0.930 indicated an excellent prediction , moreover, at an optimal cutoff point of 38 ng ml^{-1}, CD64 was 85% sensitive, 100% specific and 92.5% accurate in prediction of sepsis with a positive and negative predictive values of 100% and 87%, respectively, Figure 5 and Table 7.

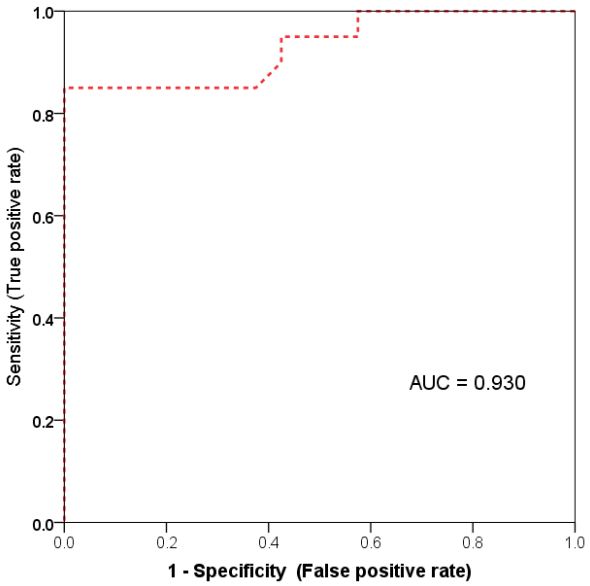

Figure 5. Receiver operating characteristics (ROC) curve analysis for the validity of CD64 as predictor of Sepsis

Table 7. Validity parameters for CD64 as predictor of Sepsis compared to controls

Validity Parameter	Value
Area under the ROC curve (AUC)	0.930
Optimal cutoff point	38 ng/ml
Sensitivity	85.0%
Specificity	100.0%
Accuracy	92.5%
Positive predictive value	100.0%
Negative predictive value	87.0%

DISCUSSION

According to the study's most general findings, most individuals with sepsis revealed the presence of Gram-negative bacteria more than Gram-positive bacteria. Other investigators have made similar observations in the world. Other researchers throughout the world have made similar findings. Gram-negative bacteria, for example, account for roughly 50-70 percent of nosocomial infections in the United States, Brazil, and Nepal, and similar data has been reported from other regions of the world [9-11].

Because of its high mortality and morbidity, as well as its financial burden, sepsis remains a problem for physicians and the health system in general [12, 13].

The most general results in this study evidenced that most patients with sepsis revealed the appearance of Gram-negative bacteria further than Gram-positive bacteria. Similar observations have been made by other investigators in the world. For instance, the type of organism causing severe sepsis is a main indicated of the result. Despite the fact that most previous studies have showed a rise in the occurrence of gram-negative organisms, the most recent European Prevalence of Infection in Intensive Care (EPIC II) study found that gram-negative organisms were more prevalent (62.2 percent). Infecting organism patterns were comparable to those seen in earlier research, with S. aureus (20.5 percent), *Pseudomonas* species (19.9%), *Enterobacteriacae* (primarily *E. coli*, 16.0 percent), and fungi being the most common (19 percent). *Acinetobacter* was found in 9% of all infections, with considerable infection rates in different parts of the world.

Early identification of infection and sepsis to begin successful treatment is linked to improved survival, and previous studies have shown that the earlier period of beginning an effective antibiotic is a greater indicator of survival and outcome in sepsis patients [14].

Furthermore, according to the World Federation of Pediatric Intensive Care and Critical Care Societies, Sepsis accounts for 60-80 percent of all deaths in the developing world each year, affecting more than 6 million newborns and children [15]. The type of bacteria that causes severe Sepsis is a major factor of prognosis [16]. Bacteria are the most common causal germs in Sepsis, according to an epidemiological study conducted in the United States from 1979 to 2000 [17]. Rapid and reliable species identification of these organisms is critical for accurate diagnosis and timely, effective treatment of severe illnesses [18, 19].

Furthermore, some strains with distinct biochemical characteristics that do not fit the description chart are commonly used in our laboratories to identify bacterial genera and species.

Using routinely available assays, laboratories have no trouble recognising typical strains of common bacteria. When atypical strains or rare or recently reported species are separated and must be classified, problems arise; these condition impacts are mistaken or unidentified strains. As a result, various commercially available automated systems have been examined for ordinary laboratory use. Many peer-reviewed articles have shown that automated VITEK 2 technology and VITEK 2 ID cards deliver reliable and accurate findings for Gram-positive cocci and Gram-negative bacilli that are clinically meaningful [20, 21]. In this study, the automated system VITEK2 (index 4-1) successfully identified the *S. typhi* isolates, *S. aureus* also identified in index (2) and *K. pneumonia* show in the index (3).

Another advantage of the VITEK 2 system is identifying various *Staphylococcus* species that cannot be identified by the conventional method. VITEK 2 shows a variety of coagulase-negative *Staphylococci* genus and species according the indexes (1), (2), (3) in appendixes.

Many types of research have noticed that the increased use of broad-spectrum antibiotics as the last two decades joined by the increasing number of immunocompromised and severely ill patients, has directed to the emergence of coagulase-negative staphylococci mainly, and these organisms perform a notable performance in nosocomial bloodstream infections [22, 23].

A neutrophil cluster of differentiation 64 (CD64) has been identified as a potential biomarker for bacterial infection and sepsis in numerous studies [21] CD64 is an Fcγ receptor revealed mainly on monocytes and, to a significantly lesser extent, on resting polymorphonuclear leukocytes (PMNs). Bacterial infection or sepsis begins to an improvement in CD64 appearance on initiated PMNs, CD64 levels on the surface of PMNs can be assessed with a flow cytometer [24].

We expect a great sensitivity and specificity biomarker to classify the septic subjects correctly to reduce the diagnosis uncertainty. Generally accepted SIRS criteria are insufficiently sensitive and particular [25, 26].

The CD64 index is a useful test for detecting and treating sepsis and other serious bacterial infections. Since many modern hematology analyzers have flow cytometers and can perform CD4 sub setting, adding CD64 index testing to these platforms would make this test even easier. Modern hematology analyzers have already proven to be capable of performing this test with accurate results, and at least two companies have developed analytic-specific reagent kits for their analyzers. This should enable even those with no prior experience with standalone flow cytometers to conduct this test.

CD64 (FcgRI) is an Fc receptor for IgG found in macrophages, monocytes, eosinophils, and neutrophils. In the presence of microbial wall components, complement split products, and several pro-inflammatory cytokines, such as granulocyte colony-stimulating factor (G-CSF) and interferon-gamma (IFN-), studies have demonstrated that CD64 expression increases during an infection [27-29].

When it came to detecting systemic infection or sepsis, CD64 had the highest sensitivity and specificity [30]. The findings backed up their previous research: Surprisingly, the cut-off level for neutrophil CD64 was much lower than in the previous study; unfortunately, no explanation was given [30, 31].

Livadi and colleagues published the first research to include critically ill patients from an intensive care unit (ICU), which represents the clinical situation where sepsis biomarkers are most likely to be useful. These researchers discovered that serum interleukin-6 (IL-6) and IL-8, as well as neutrophil CD64, were the best indicators of sepsis severity in the early stages, with CD64 and IL-8 also predicting mortality [32].

Cardelli et al. [33] conducted a more comprehensive report involving adult sepsis patients. They included 112 ICU patients with sepsis who were clinically suspected; sepsis was confirmed in 52 of these patients by a positive blood culture. With high sensitivity and precision, neutrophil CD64 was able to differentiate these patients from those in whom sepsis could not be confirmed.

CD64 also seemed to be more specific than procalcitonin. Another small research in patients suspected of sepsis was only published as an abstract with few details; however, it showed high sensitivity and specificity for identifying patients with positive blood cultures [34]. Furthermore, Hsu et al. found that neutrophil CD64 was better than procalcitonin at separating SIRS from extreme sepsis and septic shock in patients in a respiratory ICU [33, 35].

Furthermore, CD64 was discovered to be linked to mortality in this study. The findings of the most comprehensive study ever conducted .They looked at patients in the emergency room who had a suspected infection, fever, delirium, or acute hypotension of unknown origin. 416 (66%) of the 631 patients in the study were diagnosed with sepsis. found that neutrophil CD64 had a sensitivity of 66% and a specificity of 65% for diagnosing sepsis [36].

[37] two recent review articles offer a critical summary of almost all of the above studies. Unfortunately, the majority of research have been conducted on small patient populations and have serious methodological flaws. The outcomes are usually in agreement. The combined sensitivity and specificity of CD64 for detecting infection or sepsis were stated to be 79 percent and 91 percent, respectively.

The calculations in this meta-analysis, on the other hand, were done on studies with a highly variable nature. Adults and neonates were included, as were patients with sepsis, systemic infections, and local infections, indicating that neutrophil CD64 as a sepsis biomarker is not representative. When only septic adult patients are considered, the average sensitivity is 88.3 percent (95 percent confidence interval 78.1-94.1 percent), and the precision is 87.6 percent (71.8-95.2 percent) [38] .

However, combining these studies has its drawbacks. Individual studies were designed in a variety of ways. The conditions for inclusion were not always the same. They often used small numbers of patients, and there was a lot of variance in methodological methods and cut-off value description. To fully understand the clinical utility of neutrophil CD64 as a sepsis marker in an ICU environment, much larger, multicenter studies with uniform inclusion criteria and standardized analytical methods are needed [31].

According to [39], preterm newborn infants' neutrophils had a moderately increased level of CD64 expression that decreased during their first month of life to the level seen in term newborn infants and adults' neutrophils.

[40], on the other hand, had previously stated that we were unable to confirm this discrepancy in our research. The discrepancy between adults and neonates may be due to a less developed neutrophil response to gram-negative bacteria infection in neonates. Furthermore, it has been discovered that leucocytes from patients with streptococcal infection express this Fc receptor at a higher level. Streptococcus pneumonia was found in one of our patients' sepsis episodes with a very high CD64MFI value (273.2) [41].

CD64 expression has been found to be a highly specific predictor of NS, despite having poor sensitivity in one sample and according to [42] this laboratory parameter has a high specificity and positive predictive value for sepsis (96.8% and 88.8%, respectively), but a low sensitivity (25.8%) and a moderate negative predictive value for sepsis (57.4 percent). CD64 has a high diagnostic performance for sepsis (sensitivity 87.9%, specificity 71.2%) [30].

At the time of initial diagnosis, defined optimum diagnostic cutoff levels, AUCs, sensitivity and specificity of CD64MFI for neutrophils, and CD64 index as 72.0, 0.85, 65.5, 92.6 percent and 2.45, 0.83, 65.5, 88.9%, respectively, for sepsis [43]. Since the measurement of neutrophil CD64 is a quantitative flow cytometric assay, it necessitates a procedure of much more stringent standardization than other qualitative flow cytometric studies.

According to a review of the existing literature, different anti-CD64 antibodies, flow cytometers, and measured parameters for neutrophil CD64 have all been used [5, 32, 44, 45].

CONCLUSIONS

1- The current study results revealed that both sexes (males and females) were responsive to bacterial infection, and males were more responsive to infection, and the age group 30- 39 years were most affected.

2- *Salmonella typhi* bacteria is the most common bacterial causes of bacterial sepsis infection**.**

3-The high level CD64 in patients with sepsis compared with control group.

Conflict of interest

The authors declared no conflict of interest.

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