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RESEARCH ARTICLE

COMPARATIVE STUDY TO ASSESS THE EFFICACY OF EOSINOPHIL COUNT AND NEUTROPHIL-LYMPHOCYTE COUNT RATIO VERSUS PROCALCITONIN LEVELS IN PATIENTS WITH SEPSIS.

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Abstract

There is scarce evidence on the use of eosinophil count as a marker of outcome in patients with infection. The aim of the study was to evaluate whether changes in eosinophil count, as well as the neutrophil-lymphocyte count ratio (NLCR), could be used as clinical markers of outcome in patients with sepsis when compared to Procalcitonin (PCT) levels.

Methods:- We performed a prospective observational study on patients admitted in sepsis between November 2013 and September 2015. A total of 100 patients in sepsis were included. Chi square test, student t test and ANOVA were used to analyze the behavior of eosinophil count and NLCR in survivors and non survivors.

Results:- In the analysis, the main independent risk factor for mortality was persistence of an eosinophil count below 40 cells/$mm^{3.}$ An NLCR value was also an independent risk factor but was of lesser importance. The mean eosinophil count in survivors showed a tendency to increase rapidly and to achieve normal values between second and the third day. In these patients, the NLCR was <7 between the second and third day.

Conclusion:- Both sustained eosinopenia and persistence of an NLCR <7 were independent markers of mortality in patients with sepsis.

Introduction:-

Several biomarkers, such as C-reactive protein and Procalcitonin have been used to indicate bacterial infection.They have limited sensitivity and specificity, but the greatest limitation of Procalcitonin is probably its high cost, placing it practically out of reach of developing countries like India. Hence the need to evaluate Eosinophil Count and Neutrophil-Lymphocyte Ratio as an indicator of sepsis considering their lower cost and easier accessibility.[1] In an emergency care setting, both lymphocytopenia and NLCR are better predictors of bacteraemia than routine parameters like CRP level, WBC count and neutrophil count. Attention to these markers is easy to integrate in daily practice and without extra costs.[2]

Eosinopenia can be used as a diagnostic marker of sepsis in newly admitted critically ill patients. Eosinopenia is a better diagnostic marker than CRP, and may become a helpful clinical tool in ICU practices. [3] The neutrophil to

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lymphocyte count ratio is an easily measurable parameter that indicates the severity of systemic inflammation and sepsis. Moreover NLCR is a useful parameter for predicting bacteraemia in emergency care settings.[4] It is already known that eosinopenia typically accompanies theresponse to acute infection This marked reduction in thenumber of circulating eosinophil leucocytes in acute infectionwas first described by Zappert in 1893 and was utilizedduring the first quarter of the last century as a useful diagnostic sign. After the observation that eosinopenia is part of the normal response to stress, it was assumed that eosinopeniaof acute infection is a secondary response to stress caused by the infection[3].

An early diagnosis of sepsis before receivingthe results of microbial culture would certainly facilitate thechoice of antibiotic therapy and reduce the patient mortality. Unfortunately, the availability of a highly specific sensitivemarker of infection is still not satisfied. An ideal marker ofinfection would be highly specific, highly sensitive, easy tomeasure, rapid, inexpensive, and correlated with the severityand prognosis of infection[3]

Hence the need to evaluate eosinophil count as an easily available and lesser expensive marker in the prognosis of sepsis[1]. In principle, four classes of microorganisms i.e bacteria, fungi, viruses and parasites are responsible for infection, out of which majority are caused by bacteria(90%). These observations suggest that there are specific host immune responses to each pathogen mediated by various sets of pathogen associated mediators of inflammation. Mechanism involves stimulation of immune cells by components of bacterial cell wall structure (peptidoglycan, lipoproteins, lipoteichoic acid, and phenol soluble modulin)[5, 6] The ultimate result is a complex interaction between the innate immune system and microbial products resulting in a vigorous inflammatory reaction, if failing to kill the invader, may progress to severe sepsis.[7] The pathologic mechanism in sepsis which leads to multiple organ failure is a complex process. Both pro and anti-inflammatory molecules are found to be involved in this process [6,7]. The two important factors leading to organ failure are persistent vasodilatation leading to hypotension ,endothelial injury and organ dysfunction[8]

Aims and objectives:-
To establish that eosinopenia and raised neutrophil-lymphocyte ratio is a comparable marker with PCT in prognosis of sepsis.

Objectives of the study:-
1. To determine serial eosinophil counts in patients with sepsis.
2. To determine serial neutrophil-lymphocyte count ratio in patients with sepsis.
3. To determine PCT levels in patients with sepsis.
4. To establish that fall in eosinophil count and increase in neutrophil-lymphocyte ratio is comparable marker with PCT in prognosis of sepsis.

Materials and Method:-
Patients fulfilling the inclusion and exclusion criteria coming to the in-patient department of general medicine in KIMS hospital. Detailed history were taken and patients were examined thoroughly. Written informed consent was also obtained from all the patient.
- Blood routine- Hb, TC, DC and ESR.
- Neutrophil and lymphocyte count for first 3 consecutive days and then on alternate days upto one week.
- Absolute eosinophil count for first 3 consecutive days and then on alternate days upto one week.
- Urine routine and Urine for Culture/Sensitivity.
- Blood for culture and sensitivity(BACTEC).
- Pus for C/S, sputum for C/S, fluid(ascitic/pleural/CSF) for C/S wherever appropriately required.
- Serum Procalcitonin Levels.

Any other investigations will be done if required based on history and other complaints

Research Design:-
A prospective observational study was conducted by purposive sampling technique in 100 patients admitted to Department of Medicine, KIMS Hospital and Research Centre. This study was conducted for 18month from November 2013 to September 2015. Both male and female patients > 18 years of age admitted to KIMS Hospital under Department of Medicine in sepsis(accc to SIRS{ACCP-CCM} criteria) and patients willing to participate were included in the study Individuals aged nts < 18 years of age. Patients with hematological cancer, HIV infection.

Patients with bronchial asthma and other atopic disorders like hay fever, atopic dermatitis, allergic conjuctivitis. Trauma patients as eosinophilia has been associated with trauma were excluded from the study. Data obtained were analysed in terms of objectives of the study using descriptive and inferential statistics.

Statistical methods:-
The following methods of statistical analysis have been used in this study. The results for each parameter (numbers and percentages) for discrete data are presented in Table and Figure. Proportions were compared using Chi-square test of significance

Student "t' test:-
The student't' test was used to determine whether there was a statistical difference between the groups in the parameters measured.

Repeated measure One way Analysis of Variance (Anova);-
Repeated measure one way analyses of variance were used to test the difference between time points. Analysis of Variance is a technique by which the total variation is split into two parts one between groups and the other within the groups. In all the above test the "p" value of less than 0.05 was accepted as indicating statistical significance. Data analysis was carried out using Statistical Package for Social Science (SPSS ver 10.5) package.

Results:-

Total of 100 patients admitted to KIMS in sepsis were studied. Majority of the study population were in the age group between 30-60 years. The elderly population >60 years comprised of **39** people whereas the young population <30 years had the least number of sepsis patients with **12.** Males comprised of **58%** of the study population and females made up the rest of the study sample with **42%.**

Respiratory tract infection constituted majority as the source of sepsis accounting for 50% of the cases. The other commonly encountered sources among the study population were urinary tract infections, CNS infections, skin and soft tissue infections and systemic bacterial infections constituting 18%, 8%, 8%, and 8% respectively. The less common sources of infection were GI infections in 4%, systemic viral infections in 2% and cause being unknown in 2% of the study population.

Most of the patients who recovered had a moderately elevated procalcitonin level on day 1. On the other hand 6 patients who succumbed had low levels of procalcitonin. This is substantiated by the 'P' value of **0.208** which is not significant.This is possibly due to lower sensitivity and specificity of procalcitonin in viral infections.

Table 1:- Distribution of Eosinophil Count by Days According To The Outcome of the study.

Outcome		Eosinophil Count		Total	$\chi 2$ value	'p' value
		<40	>=40			
Improved	Day 1	44	24	68	61.818	***<0.001***
		64.7%	35.3%	100.0%		
	Day 2	28	40	68		
		41.2%	58.8%	100.0%		
	Day 3	10	58	68		
		14.7%	85.3%	100.0%		
	Day 5	6	62	68		
		8.8%	91.2%	100.0%		
Died	Day 1	27	5	32	0.571	0.903
		84.4%	15.6%	100.0%		
	Day 2	28	4	32		
		87.5%	12.5%	100.0%		
	Day 3	29	3	32		
		90.6%	9.4%	100.0%		
	Day 5	28	4	32		
		87.5%	12.5%	100.0%		

The above table showing the trend in variability of eosinophil count among the study subjects who improved and died. As evident from the graph among the patients who improved, eosinophil count was>40/mm^{3}in **35.3%** of the patients on day 1 which improved to **91.2%** of improved patients having eosinophil count of >40/mm^3 by day 5. This is substantiated by a 'P' value of **<0.001** which is significant. (Table 1) On the contrary among the study population who died, the eosinophil count was persistently low(<40/mm^3) in **15.6%** of thestudy subjects on day 1 and continued to be persistently low(<40/mm$^{3)}$ among them in **12.5**%of them on day 5, showing not much variability, consistent with the 'P' value of 0.903.

The trend in variability of Neutrophil to lymphocyte count ratio among the study subjects who improved and died. As evident from the graph among the patients who improved, N/L count ratio was<7in **4.4%** of the patients on day 1 which increased to **83.8%** of the improved patients having a N/L count ratio of <7 by day 5. This is substantiated by a 'P' value of **<0.001** which is significant.

On the contrary among the study population who died, the N/L count ratio was persistently >7 in **90.6%** of the study subjects on day 1 and continued to be persistently high among them with **56.3**%of them still showing N/L count ratio >7 on day 5, consistent with the 'P' value of **0.008**.

Table 2:- Comparison of mean eosinophil count with outcome of the study at different days.

Eosinophil Count

Day	outcome	N	Mean	Std. Deviation	Minimum	Maximum	't' value	'p' value
Day 1	Improved	68	36.93	25.152	4	97	1.315	0.254
	Died	32	30.16	32.105	10	180		
Day 2	Improved	68	53.53	30.318	8	140	19.356	***<0.001***
	Died	32	27.19	21.896	4	80		
Day 3	Improved	68	91.32	36.102	16	180	72.370	***<0.001***
	Died	32	24.69	37.467	6	162		
Day 5	Improved	68	112.54	44.536	10	224	82.601	***<0.001***
	Died	32	28.56	39.836	6	200		

The above table represents the mean eosinophil count on each day of study in the population who improved and in those who died. It was seen that the mean eosinophil count gradually increased over the course of five days to>40/mm3among those who improved and remained persistently low (<40/mm3) when compared with those who died. This was further substantiated by the significant 'P' value which remains <0.001 from day 2 through to day 5 denoting the importance of mean eosinophil count in the prognosis of sepsis.(TABLE 2)

Table 3:- Comparison of mean neutrophil to lymphocyte count ratio according to the outcome on different days of study

Neutrophil-Lymphocyte Count ratio

Day	outcome	N	Mean	Std. Deviation	Minimum	Maximum	't' value	'p' value
Day 1	Improved	68	23.06	13.140	6	48	3.977	0.049
	Died	32	17.47	12.937	6	48		
Day 2	Improved	68	16.12	9.133	4	41	1.976	0.163
	Died	32	13.38	9.033	4	38		
Day 3	Improved	68	8.22	4.333	3	18	9.225	***0.003***
	Died	32	11.69	7.004	4	32		
Day 5	Improved	68	5.69	3.525	2	21	21.640	***<0.001***
	Died	32	10.56	6.970	3	30		

The above table represents the mean N/L count ratio on each day of study in the population who improved and in those who died. It was seen that the mean N/L count ratio gradually decreased over the course of five days to<7among those who improved and remained persistently high (>7) when compared with those who died. This was further substantiated by the significant 'P' value which remains <0.005 from day 3 through to day 5 denoting the importance of mean N/L count ratio in the prognosis of sepsis. (TABLE 3)

The mean procalcitonin levels was elevated in both the improved study population (8.672) and those who died (9.347). The 'P' value was insignificant at 0.757.

Conclusion:-

Both sustained eosinopenia and persistence of an NLCR >7 were independent markers of mortality in patients with sepsis. Persistent eosinopenia <40 cells/$mm^3$and NLCR > 7 were found to be comparable and better markers than PCT when predicting the prognosis in sepsis.

Discussion:-

In our study out of 100 patients admitted to the hospital in severe sepsis, the mortality was 32%. In another study done Khalid Abidi and et al, [3] the mortality rates were found to be 33% and hence was comparable with our study. In our study 58% of the sample population comprised of males and the remaining constituted females. In a study done by R. Terradas and et al[1], males constituted 56.9% and females 43.1% which was similar to our study.

The mean age in our study was 46.4 years compared to the mean age of 67.7 years in the study by R. Terradas and et al[1]. The difference is probably because of regional differences and a dominance of younger and middle age groups in India. In our study, respiratory tract infections **(50%)** were found to be the most common source of septicaemia followed by urinary tract infections.

However in the study by R. Terradas[1,] urinary tract infections **(73%)** was found to be the most common source of sepsis followed by abdominal infections. The difference is probably due to a higher prevalence of repiratory tract infections and lower coverage of pneumococcal and influenza immunization in India.

In our study 40% of the patients required ionotropic support compared to only 12.2% requiring vasopressors in a study by R.Terradas and et al[1]. This difference may probably be explained by varying severities of sepsis on the day of admission between the two groups and a later approach to hospital in our group considering the poor socioeconomic background of our patients.

Diabetes and hypertension constituted 48% of the comorbities in our study. However in the study by R.Terradas[1], diabetes made up for 63% of the total comorbities. This difference is probably due to the varying prevalence of communicable and non- communicable between India and the West.

In our study PCT levels were elevated in both the groups, among those who improved and those who died. The 'P' value at **0.208** was not significant. This is comparable to other studies where the PCT was found to be elevated in all the patients. This reflects the low sensitivity and specificity of PCT especially in determining the prognosis. Another aspect being the non- reliability of PCT in viral infections.

The mean eosinophil count in our study among survivors between the second and the third day was **53.5/mm^3**, which is comparable to the mean eosinophil count of **45.6/mm^3** between the second and third day in a study done by R. Terradas[1]. An increase in eosinophil count was found to be a positive prognostic factor in our study substantiated by a 'p' value of <0.001.

This was further seconded by studies from R. Terradas[1]and Khalid Abidi[3], whose studies proved a positive co-relation between increase in eosinophil count and recovery of the patient. Both the studies had a significant 'p' value of <0.001.

N/L count ratio

Our study showed a persistence of N/L count ratio of >7 in patients who died. This was comparable with a study by R. Terradas[1] where the mean N/L count ratio was found to be **9.3** at the end of three days among non survivors. Our study had a mean N/L count ratio of **11.3** at the end of three days among non survivors and a significant 'p' value of **<0.001**.

Summary:-

This was a prospective observational study conducted on 100 patients in sepsis in KIMS hospital, Bangalore from November 2013 to September 2015. Patients admitted in sepsis were defined by ACCP/SCCM SIRS criteria. PCT

levels were sent on the first day. Serial eosinophil count and serial neutrophil to lymphocyte count ratios were determined and the patients were followed up. Blood, urine culture/ sensitivity and appropriate body fluid C/S were sent wherever necessary. The statistical analysis for the above parameters were done using chi square, student t test and anova test. The cut-offs for eosinopenia was taken as <40 cells/$mm^3$and a NLCR >7 was taken to be significant. Patients were followed up until their hospital stay (either death or discharge). Patients who survived were found to have a marked increase in eosinophil count and a decrease in NLCR by the second to third day. On the contrary, the patients who died were found to have persistently low mean eosinophil counts(<40/mm^3) and a high NLCR even at the end of five days. Persistent eosinopenia and high NLCR were found to be comparable if not better and reliable markers than PCT in the prognosis of sepsis.

Conflict of interest : The authors have no conflict of interest.

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