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Original Research Article**Evaluation of ischemia modified albumin in patients of sepsis****Sibasish Sahoo^{*1}, Saurav Patra², Tapaswini Pradhan², B Das² and A K Das²**¹Department of Biochemistry, AIIMS (Raipur) India²Department of Biochemistry, KIMS (Bhubaneswar) India***Correspondence Info:**

Dr. Sibasish Sahoo

Senior Resident,

Department of Biochemistry,

All India Institute of Medical Sciences, Raipur, India. Pin-492022

E-mail: drsibasish18@gmail.com**Abstract**

Sepsis is characterized as the combination of pathologic infection and physiological changes known collectively as the Systemic Inflammatory Response Syndrome. This response results in physiological alterations that occur at the capillary endothelial level. In the early stages, the clinical manifestations of this process are nonspecific and it is often underappreciated in clinical practices. These shortcomings in both culture and available blood tests have driven researchers to find other more specific markers. So this study was conducted to find out a suitable endogenous marker which can detect sepsis and predict its severity. A cross sectional (case control) study was carried out with 76 cases divided into two groups depending on the features of systemic inflammatory response syndrome and diagnosed cases of sepsis. We tried to evaluate the Ischemia Modified Albumin and serum CRP in septic patients and compare it with age matched normal subjects. The above parameters were also compared with physical parameters like age & sex, temperature, respiratory rate, heart rate, blood pressure & total leucocytes count.

Keywords: Ischemia Modified Albumin (IMA), C-reactive protein (CRP), Systemic Inflammatory Response Syndrome (SIRS).

1. Introduction

Septicemia is defined as the presence of bacteria or their toxin in the bloodstream[1]. The term *septicemia* is also used to refer to sepsis in general[2]. Sepsis is an unconquered challenge in medicine, affecting people of all ages and demographics. Septicemia is the second leading cause of death in non-cardiac ICU. Approximately 3,000 patients die of severe sepsis every day[3].

Sepsis is not a single disease entity but a progressive disorder. Progression to sepsis occurs in stages and the initial condition is called systemic inflammatory response syndrome or SIRS.

Systemic inflammatory response syndrome (SIRS) – A patient is said to be suffering from SIRS when there is presence of two or more of the following condition;

- a) Hyperthermia $>38^{\circ}\text{C}$ or Hypothermia $<36^{\circ}\text{C}$
- b) Tachypnea >24 breaths/min
- c) Tachycardia $\text{HR}>90$ beats/min

- d) Leucocytosis $>12000/\mu\text{l}$ or Leucopenia $<4000/\mu\text{l}$

Therefore, there are some unmet laboratory tools that can distinguish SIRS from sepsis for two reasons: 1) Early diagnosis and appropriate management of sepsis has been shown to reduce mortality and 2) Inappropriate antibiotic prescription which carries with it problem of cost and emerging antibiotics resistance may be minimized[4].

Currently available markers for screening, diagnosis, and determining the response to therapy of infection and septicemia are Procalcitonin(PCT), CRP, Interleukin, Protein C, Endocan.

Circulating levels of PCT in healthy subjects are below the limit of detection, production of PCT during inflammation correlates with both the presence of bacterial endotoxin and inflammatory cytokines[5]. Though Procalcitonin has become the most widely studied and reported putative biomarker for sepsis but studies have reported that PCT is

nonspecific and insensitive in the diagnosis of invasive fungal infection[6]. It is a costly marker and the test is not done in most laboratories. Other biochemical marker such as CRP, Interleukin, Protein C, Endocan have become less specific and not routinely done in all biochemical laboratories as they are costly.

So recent markers which are more specific, sensitive and cost effective and can be done easily in all laboratories i.e serum ischemia modified albumin.

2.1 Aims and Objective

Most of the markers are costly, non-specific and there are very few markers to detect septicemia at an early stage, so in my study I have chosen the above three parameters which have shown promising results in previous other studies and if confirmed could be very helpful and cost effective for early detection of sepsis.

2. Materials and Methods

The study protocol was approved by the ethical board of university. Informed consent was obtained from all the subjects & from their family members in case of confused severely ill patients. The study includes 3 groups. In which, GROUP I consists of 90 healthy volunteers free from any disease, GROUP II - 31 patients with systematic inflammatory response syndrome (SIRS) in ICU & in GROUP III - 45 septicemia diagnosed patients from ICU.

The details of the patient's clinical data which include the time of presentation at the emergency department were entered in a specialized Proforma made for the purpose of the study. Both patients with systematic inflammatory response syndrome (SIRS) in ICU & septicemia diagnosed patients from ICU were selected from a hospital having an established ICU facility. The present study was carried out in the department of Biochemistry, along with the collaboration of Intensive Care Unit of Kalinga Institute of Medical Sciences (KIMS), KiiT University, Bhubaneswar, from November 2011 to January 2013. The recruitment was done within the first 24 hrs of admission to the ICU or within 24 hrs of onset of sepsis prior to any antibiotics infusion.

2.1 Inclusion criteria

Septic subjects were recruited based on the American College of Chest Physicians/Society of Critical Care Medicine consensus criteria of 1992 for severe sepsis.

2.2 Exclusion Criteria for Sepsis Subjects:

- Age <18 and >60

- Chest pain with ST elevation myocardial infarction or dynamic ST changes on ECG
- Pregnant women
- Drug abuse
- Immunosuppressive therapy
- Severe Liver disease
- Severe hypoalbuminemia

2.3 Methodology

Routine biochemical parameters include: Fasting blood glucose; Serum urea; Serum creatinine; Total protein; Serum albumin; Liver function test; Serum CRP

Special parameters are: Serum Ischemia Modified Albumin (IMA)

All the analysis was estimated by Semi Autoanalyzer (PHOTOMETER 5010)

2.4 Sample Collection

After obtaining informed consent of the patients, blood samples were collected for routine and specific tests.

2.5 Collection of blood Sample

5 ml of blood was collected from all enrolled patients who were not given therapy for septicemia for assessment of IMA, MDA and NO products. Biochemical parameters were evaluated after separation of the serum.

A detailed physical examination of the patients were carried out such as height, weight, body built, pallor, icterus, cyanosis, thyroid gland, edema, lymph node enlargement, temperature, pulse, blood pressure, systemic examination such as CVS, CNS and Respiratory Examination.

Above said routine tests were performed of each patients on their respective blood sample after physical examination.

2.6 Estimation of serum ischemia modified albumin level (Bar Or et al 2000)

IMA is measured by an indirect method based on the albumin cobalt binding (ACB) colorimetric assay. Serum IMA was measured by addition of a known amount of Co^{2+} ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 1g/l, sigma) to serum specimen and further assessment of the unbound Co^{2+} by colorimetric assay using dithioeritol (DTT, 3g/l, sigma) for colour development, as previously described by Bar-Or et al[7].

2.7. Principle

Human serum albumin has an inherent property of binding with metals like cobalt & nickel etc at its N-terminal region. But during ischemia, the N-terminal portion of albumin is affected due to acetylation /depletion of one or more amino acids &

result in ischemia modified albumin (IMA). Ischemia modified albumin loses its ability to bind cobalt and other metals. Cobalt happens to be an indicator in this assay. Here known amount of cobalt is added to serum sample & the unbound cobalt is measured by the intensity of coloured complex formed after reacting with dithiothreitol by spectrophotometer.

2.8. Statistical analysis

Results are presented as Mean \pm SD. Statistical significance and difference from control and test values were evaluated by Z-test and F-Test was used for comparison of variance. Student's unpaired t-test was not applied due to large sample size. It is evident from various opinions of eminent statisticians that t-test should be applied to calculate p value when the sample size is less than 30. Z test is more accurate when the study is done on a large sample size. P-value of P<0.01 and P<0.05 were considered significant. Correlation coefficient was used to describe the effects of independent and dependent variables by Pearson correlation test. All statistical analyses were done using Microsoft Excel for Windows VII version, SPSS (Statistical Package for Social Sciences) and GraphPad Prism 6.0 version.

3. Results

Serum IMA, CRP, and white blood cell count were significantly higher in the sepsis group before treatment when compared with the control group. There was significant correlation between serum CRP and serum IMA ($r=0.48$, $p<0.0001$). In addition, the level of IMA shows positively correlation when compared with white blood cell count & CRP with SIRS group. A significant correlation was seen between all the parameters.

Table No-1: Number of groups and details

Group	Details	Strength
Control	Healthy volunteers free from any disease	90
I	Patients with systematic inflammatory response syndrome (SIRS) in ICU.	31
II	Septicemia diagnosed patient from ICU	45

The above table shows number of groups in this study, details of each group and strength of each group.

The first group is the control group consisting of healthy volunteers free from any disease.

The second category is group I consisting of 31 patients, this group comprises of patients admitted

in ICU with systematic inflammatory response syndrome (SIRS).

The third category is group II of strength 45, septicemia diagnosed patient in Intensive Care Unit.

Table- 2: Age and sex distribution in different groups

	Control (n=90)	Group I (n=31)	Group II (n=45)
Age (in yrs)	41 \pm 11	45 \pm 7	45 \pm 10
Male	51	19	24
Female	39	11	21

The above table represents the age and sex distribution in control group, group I and group II.

The strength of control group, group I and group II were 90, 31 and 45 respectively.

The mean age in years of the Control Group was 41 \pm 11, Group I was 45 \pm 7 & Group II was 45 \pm 10.

The number of males in Control Group was 51, in Group I it was 19 & in Group II it was 24.

The number of females in Control Group, Group I and Group II was 39, 11 and 21 respectively

Table 3: Blood pressure in different groups

	Control	Group I	Group II
Systolic BP	114.46 \pm 6.74	114 \pm 15.9	102 \pm 8.06
Diasystolic BP	75.6 \pm 7.56	76.3 \pm 12	70 \pm 9

The mean of Blood Pressure (both Systolic & Diastolic) in control, Group I & Group II were 114/75 mm/hg, 114/76 mm/hg & 102/70 mm/hg respectively. There is a decline in the mean of blood pressure in group II when compared with the control group and group I.

Table – 4: Comparisons between control and case

Parameters	Control	Case	P value	Remarks
IMA	45.19 \pm 6.39	92.52 \pm 21.85	0.0001	HS
CRP	0.28 \pm 0.09	11.08 \pm 10.6	0.0001	HS
TEMP	98 \pm 0	100.65 \pm 1.31	0.04	S
PR	72.93 \pm 3.11	87.69 \pm 13.78	0.0001	HS
RR	19.16 \pm 2.24	21.80 \pm 3.56	0.242	NS
TLC	7563 \pm 1279	17218 \pm 4408	0.0001	HS

NS-Not significant, S-Significant, HS-Highly Significant

Biochemical marker like serum IMA, & CRP show the very significant p value when compared between control group and case group i.e $p<0.01$. General Parameters like Respiratory rate was not significant i.e $p>0.05$. However Pulse rate and Total leucocyte count were highly significant $p<0.01$ and Temperature was significant i.e; $p<0.05$

Table – 5: Comparisons between control and group 1

Parameters	Control	Group 1	p value	Remarks
IMA	45.19± 6.39	71.61± 9.58	0.0001	HS
CRP	0.28± 0.09	0.92± 0.37	0.08	NS
TEMP	98 ± 0	100 ± 1.6	0.21	NS
PR	72.93± 3.11	81 ± 15	0.0096	HS
RR	19.16± 2.24	23 ± 4	0.8	NS
TLC	7563± 1279	14737± 2580	0.0001	HS

NS-Not significant, S-Significant, HS-Highly Significant

Biochemical marker like serum IMA show the very significant p value when compared between control group & group 1 i.e p<0.01. Serum CRP does not show any significant p value i.e; p>0.05. General Parameters like Temperature and Respiratory rate were not significant i.e p>0.05. However Pulse rate and Total leucocyte count were highly significant p<0.01

Table – 6: Comparisons between control and Group II

Parameters	Control	Group II	p Value	Remarks
IMA	45.19± 6.39	106.90 ± 8.48	0.0001	HS
CRP	0.28 ± 0.09	18.09 ± 8.48	0.0001	HS
TEMP	98 ± 0	101 ± 1.2	0.01	HS
PR	72.93± 3.11	94 ± 9.9	0.0001	HS
RR	19.16± 2.24	21 ± 3	0.41	NS
TLC	7563± 1279	18928 ± 4610	0.0001	HS

NS-Not significant, S-Significant, HS-Highly Significant

Biochemical marker like serum IMA & CRP show the very significant p value when compared between control group & group II i.e p<0.01. General Parameters like Respiratory rate was not significant i.e p>0.05. However Pulse rate, Temperature and Total leucocyte count were highly significant p<0.01.

Table – 7: Comparisons between group I and group II

Parameters	Group I	Group II	p value	Remarks
IMA	71.61± 9.58	106.90± 8.48	0.0002	HS
CRP	0.92± 0.37	18.09± 8.48	0.04	S
TEMP	100 ± 1.6	101 ± 1.2	0.40	NS
PR	81 ± 15	94 ± 9.9	0.38	NS
RR	23 ± 4	21 ± 3	0.61	NS
TLC	14737± 2580	18928± 4610	0.10	NS

NS-Not significant, S-Significant, HS-Highly Significant

Biochemical marker like serum IMA & CRP show the significant p value when compared between control group I & group II i.e p<0.05. General Parameters like Temperature, Pulse rate, Total leucocyte count and Respiratory rate were not significant i.e p>0.05.

Table No-8: Comparisons of variable (control with different groups) by applying Z test

Parameter		Group A	Group B	Group C	Group D
		Control VS case	Control VS SIRS	Control VS Sepsis	SIRS VS Sepsis
IMA	Z	7.40	4.13	9.65	3.68
	P	0.0001**	0.0001**	0.0001**	0.0002**
CRP	Z	120	-1.7	197.88	-2.04
	P	0.0001**	0.08	0.0001**	0.04*
TEMP	Z	-2.02	-1.25	-2.50	-0.833
	P	0.04*	0.21	0.012**	0.404
TLC	Z	7.54	5.60	8.88	1.62
	P	0.0001*	0.0001**	0.0001**	0.10
PR	Z	4.74	2.59	6.77	0.86
	P	0.0001**	0.0096**	0.0001**	0.38
RR	z	1.17	1.71	0.821	-0.50
	p	0.24	0.08	0.41	0.61

Significant*, Highly significant**

The above table shows the significant z value and p value of the biochemicals and general physical parameters of septicemia. The p values were highly significant in all the four groups for serum IMA. General parameters like Temperature, Total leucocyte count, Pulse rate and Respiratory rate were

not significant when compared between the Group I (SIRS) and Group II (SEPSIS). More ever Temperature and serum CRP was also not significant when compared between the control group and the Group I. Respiratory rates were not significant in any of the groups.

Figure No-1: Mean of Ages in study groups

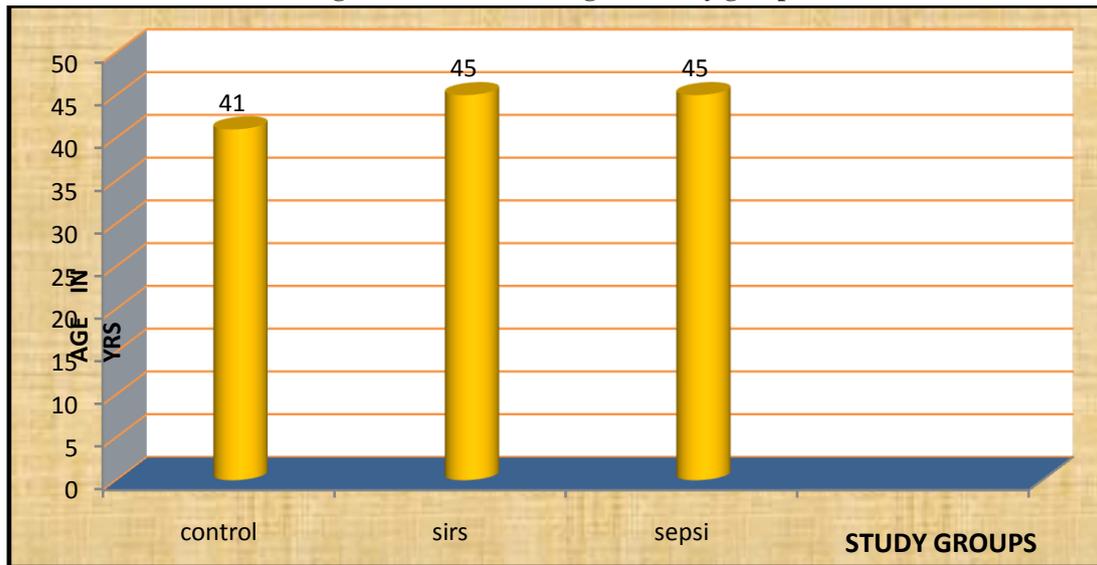


Figure No-2: Number of Males & Females in the study groups

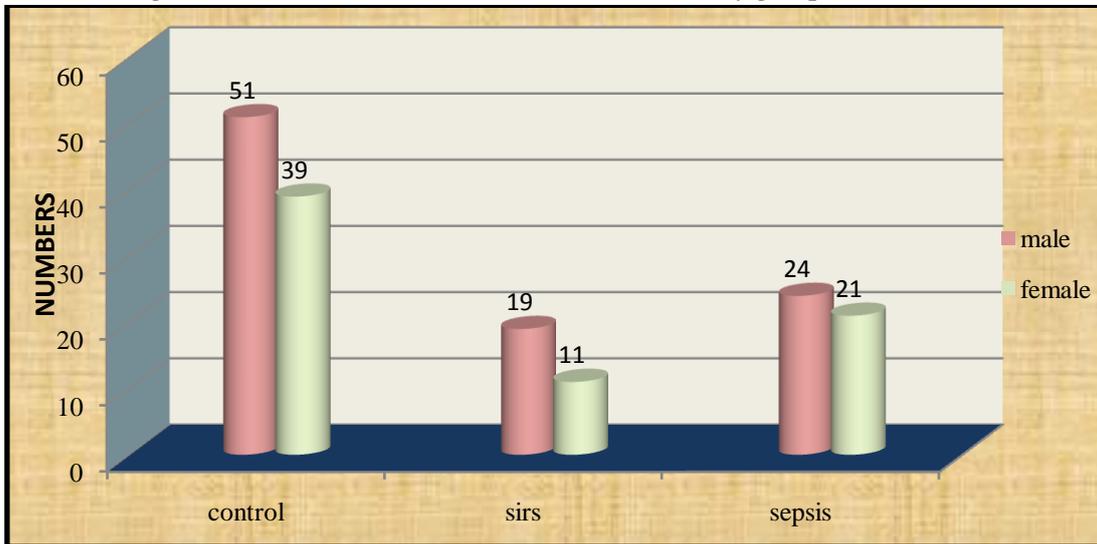


Figure No-3: Mean of Blood Pressure (Systole & Diastole) in different study groups

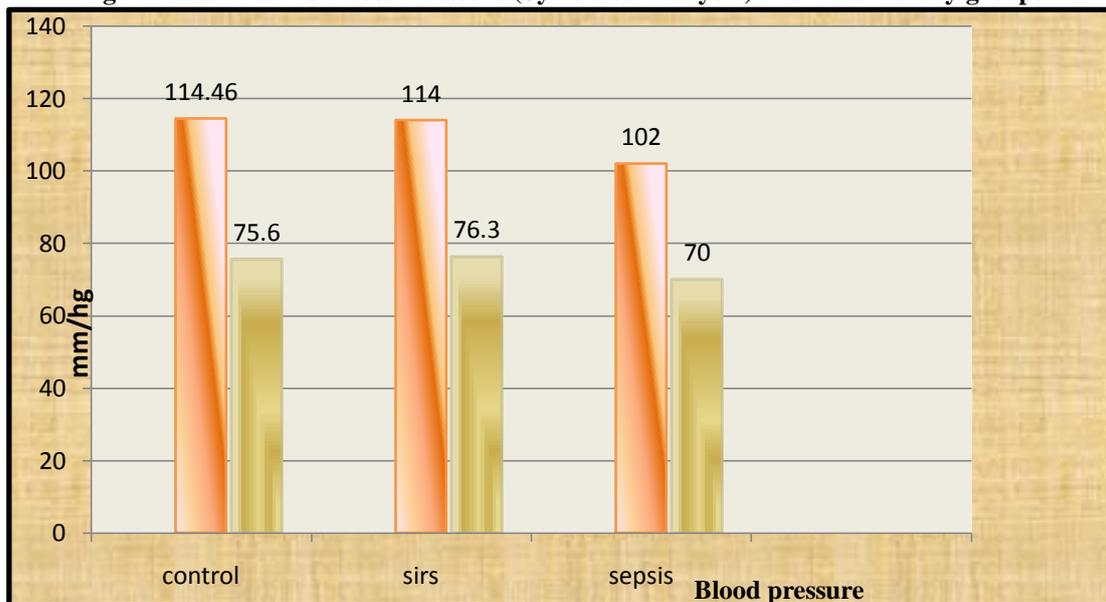


Figure-4: Mean of Respiratory Rate in different study groups.

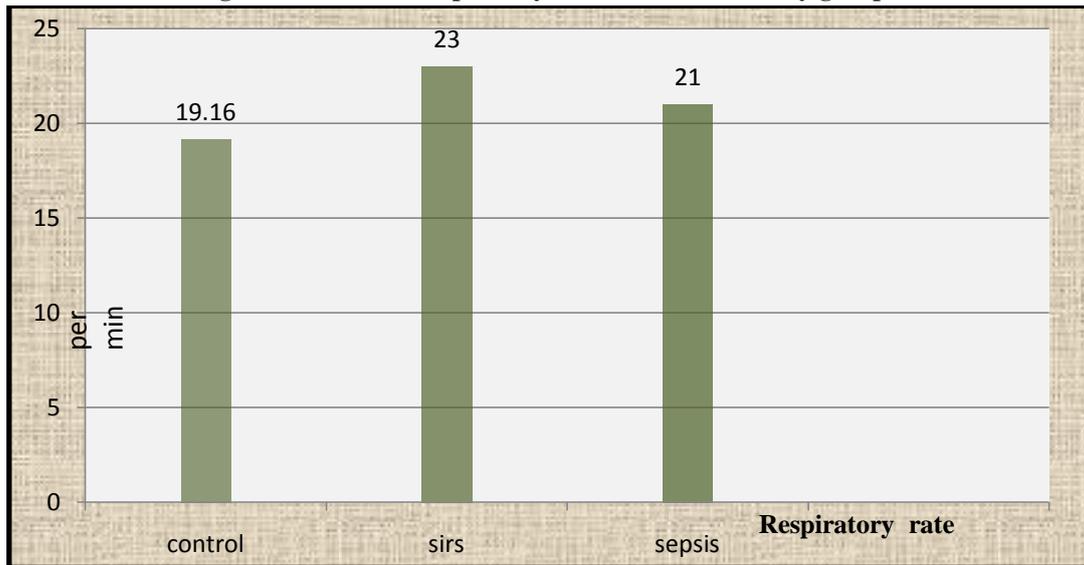


Figure-5: Mean of Heart Rate in different study groups.

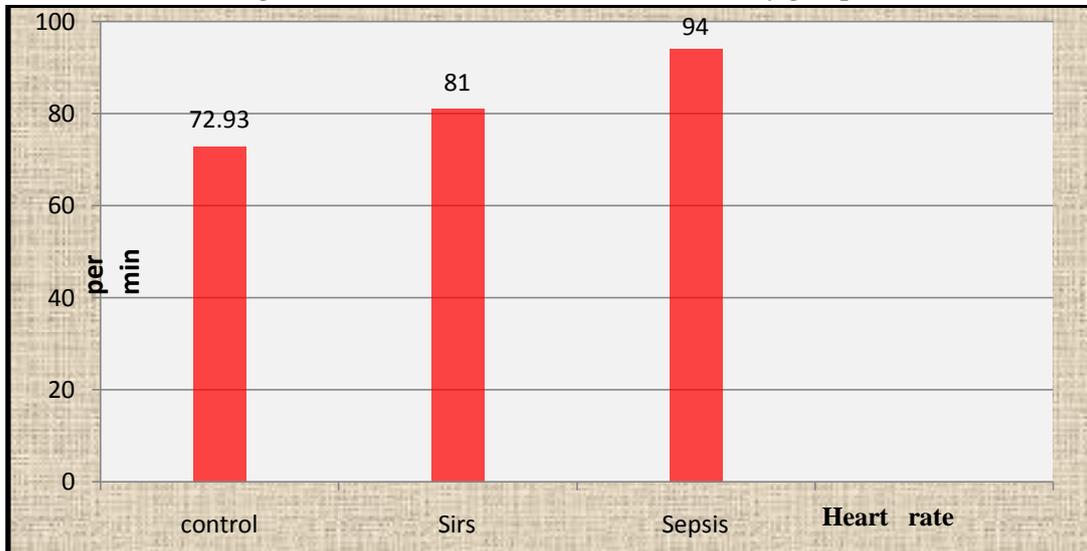


Figure- 6: Mean of Temperature in Fahrenheit in different groups.

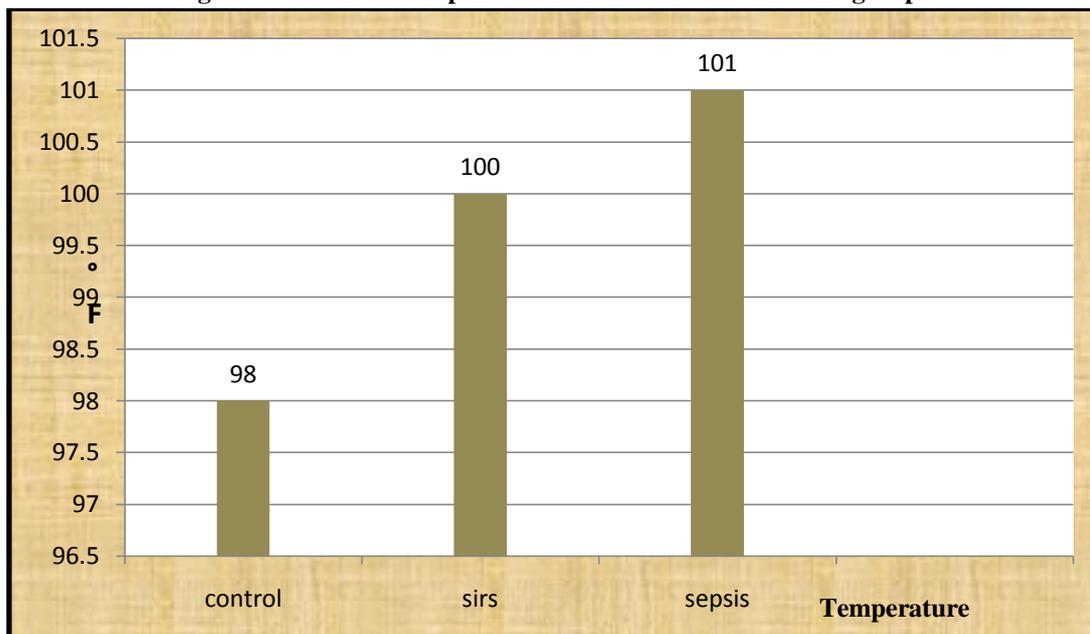


Figure- 7: Mean of Total Leucocytes Count in different groups.

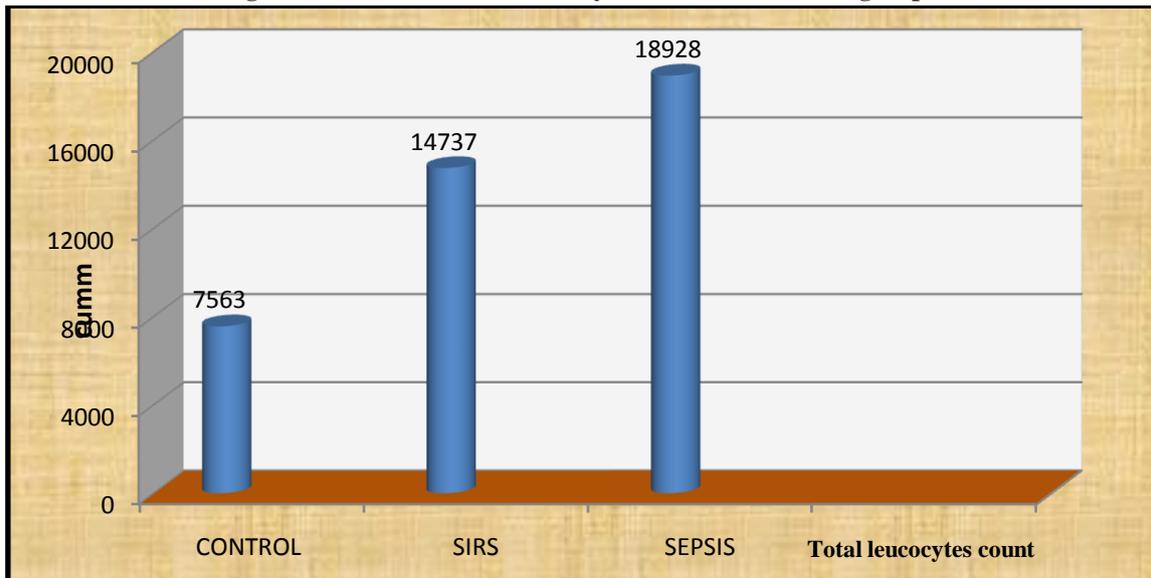


Figure- 8: Mean of Serum C Reactive Protein in different study groups.

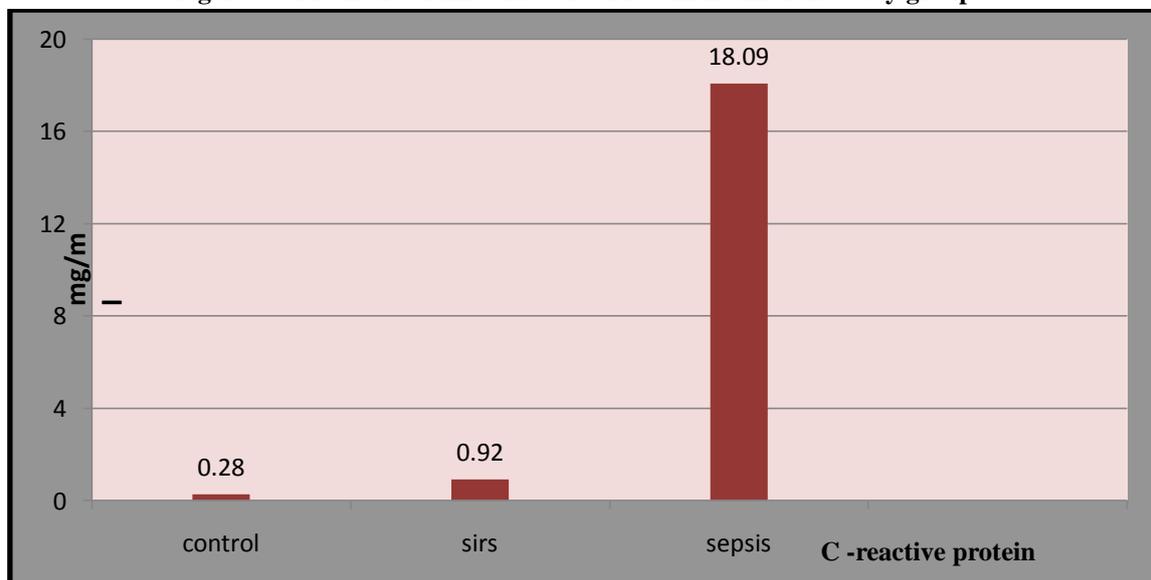


Figure-9: Mean of Serum Ischemia Modified Albumin in different study groups

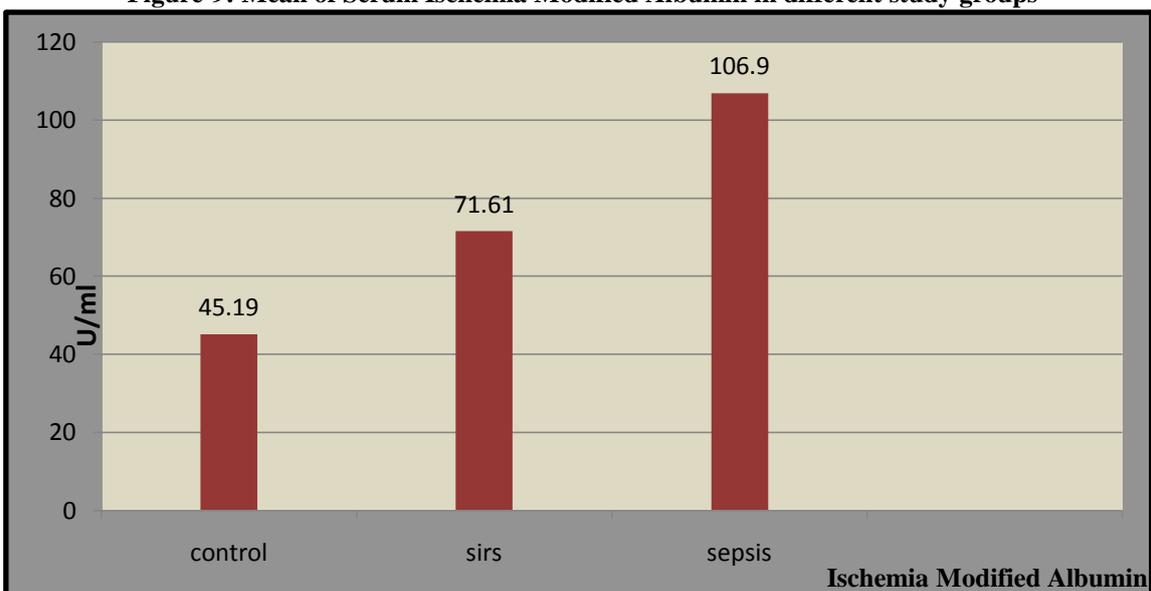
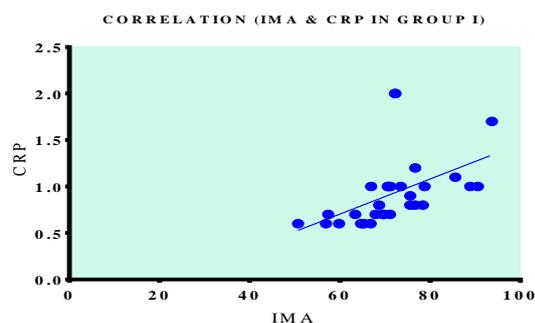
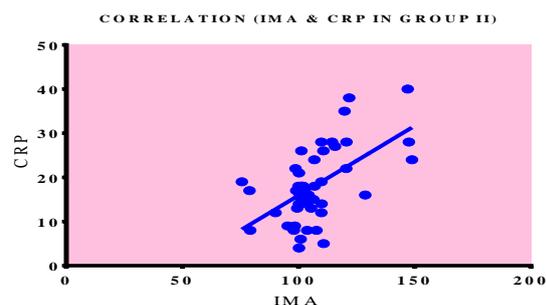


Figure 10: Correlation (IMA & CRP in Group I)**Figure 11: Correlation (IMA & CRP in Group II)**

4. Discussion

However, early recognition of this syndrome is vital in reducing mortality in sepsis. From clinical studies sepsis can be seen as a continuum of severity that begins with an infection, followed in some cases by sepsis, severe sepsis – with organ dysfunction – and septic shock. There has been a substantial increase in the incidence of sepsis during the last decades, and it appears to be rising over time, with an increasing number of deaths (Bone, 1992)[8]. Advanced age, underlying co morbidities and number of organ dysfunction are factors which are consistently associated with higher mortality in severe sepsis and septic shock.

As most cases of sepsis are associated with bacterial and fungal infection, blood culture is often considered the gold standard for diagnosis of infection. However, it lacks specificity and sensitivity. Nevertheless, current guidelines recommend culture for all the patients suspected of having sepsis, with the caveat that more than 50% of culture may be negative in the patients who have severe sepsis or septic shock. In addition, there is a substantial time delay of at least 24 hours for a positive culture report and 5 days for a negative culture report. This delay hinders the clinician's ability to make informed treatment decision in a situation that requires immediate action.

Nora Hofer *et al* concluded in their study that CRP was most widely available, most studied

and most used laboratory tests for neonatal bacterial infection and despite the continuing emergence of new infection markers it still plays a central role in the diagnosis of early onset sepsis of the neonates[9]. CRP has the advantage of being well characterized in numerous studies and was found to be the best marker to diagnose sepsis in neonates[10]. Povia P *et al* also described that CRP is useful in detection of sepsis and it is more sensitive than currently used markers such as BT and WBC[11].

In the acute phase, the role of the clinical microbiology laboratory is usually marginal, as clinicians are aware that at least 24 to 72 hrs are necessary for the confirmation of an infectious etiology, identification of the pathogen, and evaluation of its antimicrobial susceptibility. The role of blood cultures is crucial for the correct fine-tuning of antibiotic therapy. However, several factors such as empirical antibiotic therapy initiated before blood sampling or the presence of fastidious pathogens may have a negative impact on the diagnostic yield of blood cultures even when a bloodstream infection is strongly suspected.

Traditional markers of systemic inflammation such as C-reactive protein and WBC count also have proven to be of limited utility in identifying ill patients who require antimicrobial therapy. The sensitivity and specificity of these measurements for bacterial infections are low.

Severe sepsis and septic shock have a significant and increasing impact on public health, and are one of the leading causes of mortality. The definitions of sepsis syndromes established in 1992 and 2001 have contributed to improve not only epidemiological research, but also bedside diagnosis.

- ❖ The level of Ischemia Modified Albumin, which is an established marker of ischemia, was found to be significantly raised in both SIRS and sepsis patients, with a greater rise seen in sepsis.
- ❖ Ischemia Modified Albumin also assesses the severity of sepsis when correlates with APACHE II scoring methods which is preferably used by the physician and anesthetists to diagnose sepsis and its severity clinically.
- ❖ Serum Ischemia Modified Albumin showed a positive correlation in total patients group. Between SIRS and Septic patients there were significant differences in these three parameters.
- ❖ In this study Serum C-Reactive Protein had positive correlation to Serum Ischemia Modified Albumin levels in total patients group.

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