

Study of Serum HDL Levels in Severe Sepsis Patients in Medical Intensive Care Unit

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Abstract- Background: Sepsis, defined by an expert consensus definition as, the development of the systemic inflammatory response syndrome in presence of infection. (1) The current incidence of sepsis is at least 240 patients per 100,000 people in the United States population, whereas for severe sepsis it is between 51 and 95 patients per 100,000 people. The incidence rate for sepsis has been increasing over the past two decades, driving an increase in the number of deaths despite a decline in case-fatality rates. Sepsis is the tenth leading cause of death in the United States and accounts for more than 17 billion dollars in direct healthcare expenditures. (2) Sepsis has a high mortality rate, with estimates ranging from 20 to 50 percent. (3, 4) Mortality rates increase stepwise according to disease severity. In one study, the mortality rate of SIRS, sepsis, severe sepsis, and septic shock was 7, 16, 20, and 46 percent, respectively. (5)

Sepsis is one of the leading causes of death in the world. Delay in diagnosis and treatment may lead to septic shock, multi-organ failure and death. Persons with a weakened immune system are prone to develop sepsis, but the detrimental processes that may ultimately lead to the death of the patient are mostly caused by an exaggerated systemic response to an infection.

In 1993 Levine DM et al was the first to explain the protective effect of HDL-C against bacterial endotoxin. They showed, transgenic mice with high HDL-C had high levels of endotoxin bound to HDL-C, low levels of cytokine response and improved survival compared with mice having low HDL-C level.(6)

Lipoproteins have been implicated to play a role in innate immunity. (7) Knowledge of variations in blood lipid levels in patients with sepsis dates to 1980's, when studies showed significantly low HDL-C levels with sepsis, which improved with improvement in sepsis. But studies lacked correlation of with severity of sepsis with decrease in HDL-C levels nor infections agent or underlying illness. (8)

METHODS:

- Study was a prospective cohort study conducted at Kasturba Hospital, Manipal.
- Total 111 patients of severe sepsis with varied diagnosis fulfilling the inclusion criteria were included in the study.
- **STUDY PERIOD:** Study was conducted from November 2012 to August 2014.
- Institutional ethical committee clearance was obtained.

FINDINGS:

- From our study it is observed that

1. There is significant association of low HDL value on day 1 with mortality.
2. HDL value of 9 mg/dl at admission had sensitivity of 89% and specificity of 73% predicting the overall mortality in patients with severe sepsis.
3. Risk of death in patients with day 1 HDL value <10mg/dl is 7.01 times the risk of death in patients with day 1 HDL value ≥10mg/dl [95% confidence interval 2.46 - 21.81].
4. Trend of HDL correlated with clinical outcome of patients. Raising trend favours improvement in clinical condition and decreasing trend implied worsening of the clinical condition.
5. Baseline HDL value correlated with APACHE 2 score in predicting mortality in ICU Patients.

INTERPRETATION:

HDL cholesterol on day of admission can be viewed as a significant predictor of mortality in patients with severe sepsis in medical ICU patients.

FUNDING: None

I. INTRODUCTION

Sepsis is defined as the presence (probable or documented) of infection together with systemic manifestations. (9)

The incidence of sepsis and the number of sepsis-related deaths are increasing, although the overall mortality rate among patients with sepsis is declining. There are also disparities among races and between men and women in the incidence of sepsis. Gram positive bacteria and fungal organisms are increasingly common causes of sepsis. (1)

The incidence of severe sepsis in India was 16.45% of all admissions. Mean age of the population was 58.17 years (SD 18.66), of which 57.71% were male. The median APACHE II score was 13 (IQR 13 to 14) with predominant (90.93%) medical admission. Intensive therapy unit mortality of all admissions was 12.08% and that of severe sepsis was 59.26%. Hospital mortality and 28-day mortality of severe sepsis were 65.2% and 64.6%, respectively. Median duration of stay in the ICU for the severe sepsis cohort who survived was 13 days (IQR 11 to 17). The number of episodes where infection was the primary reason for

admission to the ITU (Intensive Therapy Units) was 86.32%. Culture positivity was found in 61.6%. The lung was the predominant source of sepsis (57.45 %). (10)

In 1993 Levine DM et al was the first to explain the protective effect of HDL-C against bacterial endotoxin. They showed, transgenic mice with high HDL-C had high levels of endotoxin bound to HDL-C, low levels of cytokine response and improved survival compared with mice having low HDL-C level.(6)

Lipoproteins are macromolecular complexes used by the body to transport lipid-rich molecules. (11)

Lipoproteins are classified into five groups according to their relative density:

Chylomicrons

Very low density lipoprotein (VLDL)

Intermediate density lipoprotein (IDL)

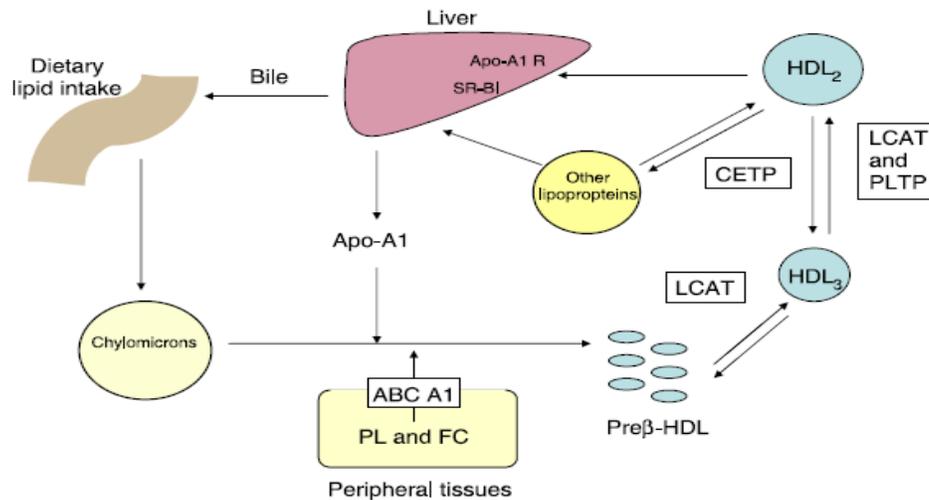
Low density lipoprotein (LDL) and

High density lipoprotein (HDL)

HDL CHOLESTEROL: HDL is a heterogeneous group of lipoproteins, varying in both composition and size, falling into a density range between 1.063 and 1.21 g/ml.(12) The major Apo protein present in HDL that provides structural stability to the spherical molecule is Apo lipoprotein A1 (Apo- A1).(12) Primary function of HDL-C is reverse cholesterol transport. Lipid-free or low-lipid Apo proteins (for example, Apo-A1) released from the liver combine with lipids derived from dietary intake (chylomicrons). These accept phospholipids and excess free cholesterol from peripheral tissues in a process promoted by the pore-forming protein ATP-binding cassette A1 transporter, forming disk-shaped pre- β -HDL.

Free cholesterol is esterified by lecithin cholesterol acyltransferase (LCAT) and the complex transforms into a spherical structure, HDL₃. HDL₃ molecules continue to engulf additional lipid molecules and Apo proteins, thereby forming mature HDL₂. (13, 14)(Figure 1)

Figure 1: Shows reverse cholesterol transport and maturation of HDL-C.



LIPOPROTEINS IN SEPSIS:

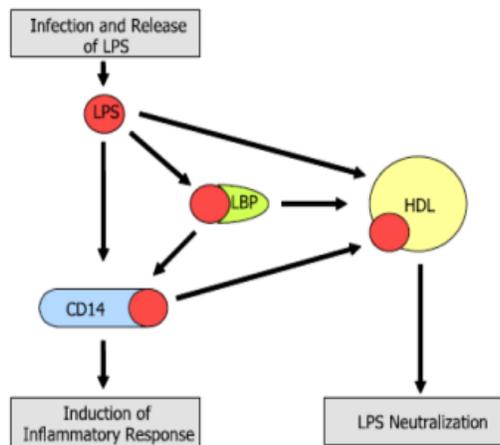
Lipoproteins have been implicated to play a role in innate immunity.(7) Knowledge of variations in blood lipid levels in patients with sepsis dates to 1980's, when studies showed significantly low HDL-C levels with sepsis, which improved with improvement in sepsis. But studies lacked correlation of with severity of sepsis with decrease in HDL-C levels nor infections agent or underlying illness.(8)

Mechanisms of low HDL in severe sepsis are multifactorial:

- 1) Neutralizing of toxic bacterial substances

Figure 2: Binding of LPS to LBP or sCD14 (soluble CD14) then being transferred to HDL.

Binding and neutralization of LPS by HDL



- 2) Suppression of lipoprotein synthesis and LCAT activity
- 3) Rapid clearance of LPS bound HDL-C
- 4) Decreased activity of ABC 1 activity
- 5) Increased Human secretory phospholipase A2 (sPLA2) activity
- 6) Dilutional effect due to resuscitation fluids.

II. MATERIALS AND METHODS

- Current study was a prospective cohort study conducted at Kasturba Hospital, Manipal.
- Total 111 patients of severe sepsis with varied diagnosis fulfilling the inclusion criteria were included in the study.

STUDY PERIOD: Study was conducted from November 2012 to August 2014.

The following inclusion and exclusion criteria were used:

INCLUSION CRITERIA:

- Patients with age greater than 18 years and satisfying the criteria for severe sepsis according to International guidelines for management of severe sepsis and septic shock: 2012(10), were included in the study and not received parenteral antibiotics in outside hospitals.

EXCLUSION CRITERIA:

- Patients on treatment with statins
- Chronic liver disease, chronic kidney disease, thyroid dysfunction, diabetes, severe anaemia and malignancy
- Patients with known chronic inflammatory condition like Human immunodeficiency virus disease, SLE (Systemic lupus erythematosus) and RA (Rheumatoid arthritis)

- Patients diagnosed to have malabsorption disorders
- Patients who were discharged against medical advise.

METHODOLOGY:

- A total of 111 patients satisfying inclusion criteria were included in our study after obtaining informed consent.
- All the patients were followed prospectively during their entire course of stay in the hospital. Serum HDL levels were done on the day of admission and repeated on day 5. APACHE 2 score was calculated for all patients on day of admission to estimate the risk of death.
- Early morning serum samples were collected on day 1 of admission and day 5 for HDL cholesterol measurement. Cholesterol measurement was done by ROCHE COBAS C systems clinical chemistry automated analyser. The concentration of HDL cholesterol was measured after precipitation of beta lipoproteins with dextran sulfate and magnesium chloride.

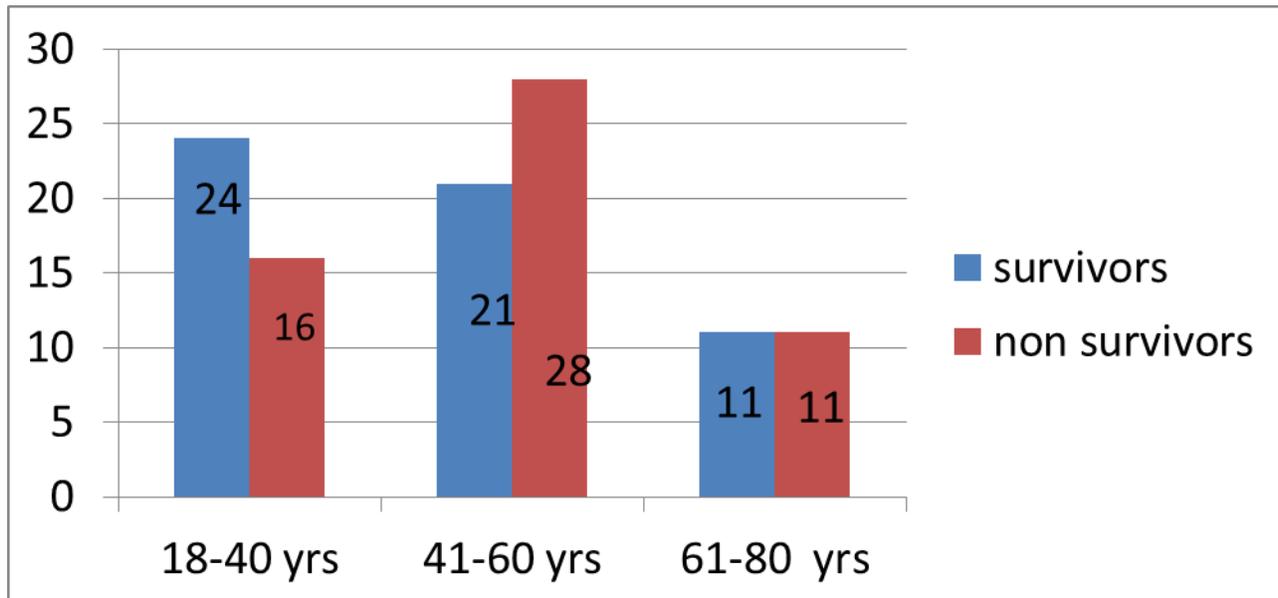
III. OBSERVATIONS AND ANALYSIS

- Current observational study included 111 patients with varied disease presentations admitted to medical intensive care unit with diagnosis of severe sepsis according to International guidelines for management of severe sepsis and septic shock: 2012(10).

Table 1: Shows baseline characteristics of survivors and non survivors.

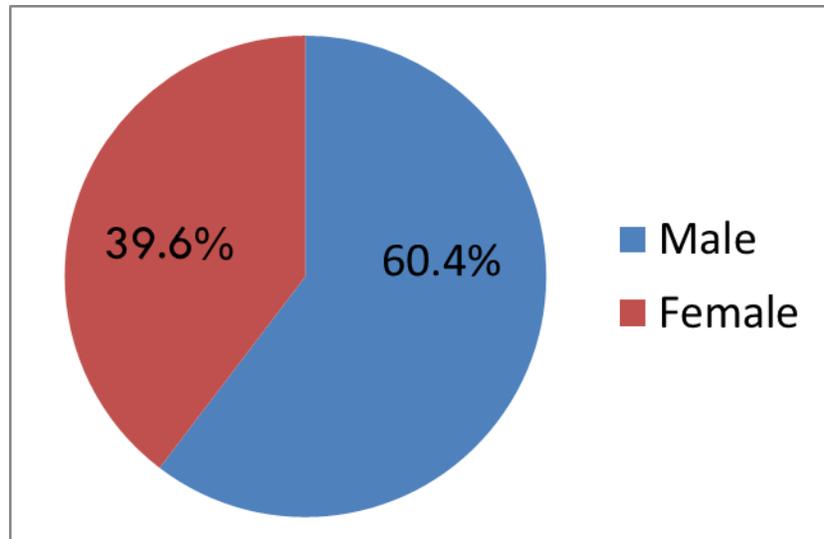
Characteristic	All patients(n=111)	Survivors (n=56)	Non survivors (n=55)	P value
Age (yr)	47.58+/-14.99	46.04+/-14.66	49.15+/-15.29	
Males	67 (60.4%)	28 (50%)	39 (70.9%)	
Females	44 (39.6%)	28 (50%)	16 (29.1%)	
APACHE II score	16 (11-21)	14 (8-18)	22 (17-27)	<0.003
Albumin	2.7 (2.2-3)	2.65 (2.2-3)	2.7 (2.4-3)	NS
Bilirubin	3.5 (1.6-5.9)	2.85 (1.02-6.17)	3.7 (2.0-5.6)	NS
INR	1.18 (1.02-1.49)	1.13 (1.02-1.36)	1.23 (1-1.6)	NS
Positive blood culture	17	6	11	NS
MODS	78 (70.3%)	34 (43.6%)	44 (56.4%)	NS
Thrombocytopenia	72 (64.86%)	35(48.6%)	37 (51.4%)	NS
Acute kidney injury	69 (62.16%)	31(45%)	38 (55%)	NS
Hypotension	54 (62.16%)	18 (33%)	36 (67%)	<0.002
Acute lung injury	89 (80.18%)	40 (45%)	49 (55%)	NS
Dialysis	35 (31.53%)	18 (51%)	17 (49%)	NS
Gram negative bacteria	26	11	15	NS
Gram positive bacteria	5	3	2	NS
Scrub typhus	21	12	9	NS
Leptospirosis	8	3	5	NS
Dengue	2	2	0	
Malaria	1	0	1	

Figure 3: Age distribution



Maximum number of people 49 out of 111 (44.1%) were in the age group of 41-60 yrs.

Figure 4: Gender distribution



Our study had total of 111 patients, of which 67(60.4%) were males and 44(39.6%) were female.

Others parameters like presence of acute lung injury, acute kidney injury, acute liver Injury (raised bilirubin levels and coagulopathy), baseline platelet count, baseline serum albumin levels and frequency of dialysis used as renal replacement therapy were comparable in numbers among survivors and non survivors and not found to have effect on mortality to reach statistical significance.

Low HDL at admission and association with mortality in severe sepsis:

In current study low HDL levels were noted in patients with severe sepsis so, we studied the association of day of admission HDL with mortality.

Table 2: Association between day1 HDL and survival.

		FREQUENCY	Mean HDL VALUE	Std. Deviation
Day 1 HDL	Non survivors	55	5.76	2.937
	survivors	56	11.79	5.714

P<0.001, there is significant difference in DAY 1 HDL value between survivors and non survivors. Independent samples T test.

Relative risk of death: After noting the significant difference of mean HDL between survivors and non survivors in current study, we calculated the relative risk of death among two groups HDL<10 mg/dl and HDL≥10 mg/dl on day of admission.

Table 3: Comparison of mortality between HDL≤10mg/dl and HDL≥11mg/dl

		survivors	Non survivors	Total
Day 1 HDL	<10mg/dl	21	50	71
		29.6%	70.4%	100.0%
Day 1 HDL	≥10 mg/dl	35	5	40
		87.5%	12.5%	100.0%
Total		56	55	111
		50.5%	49.5%	100.0%

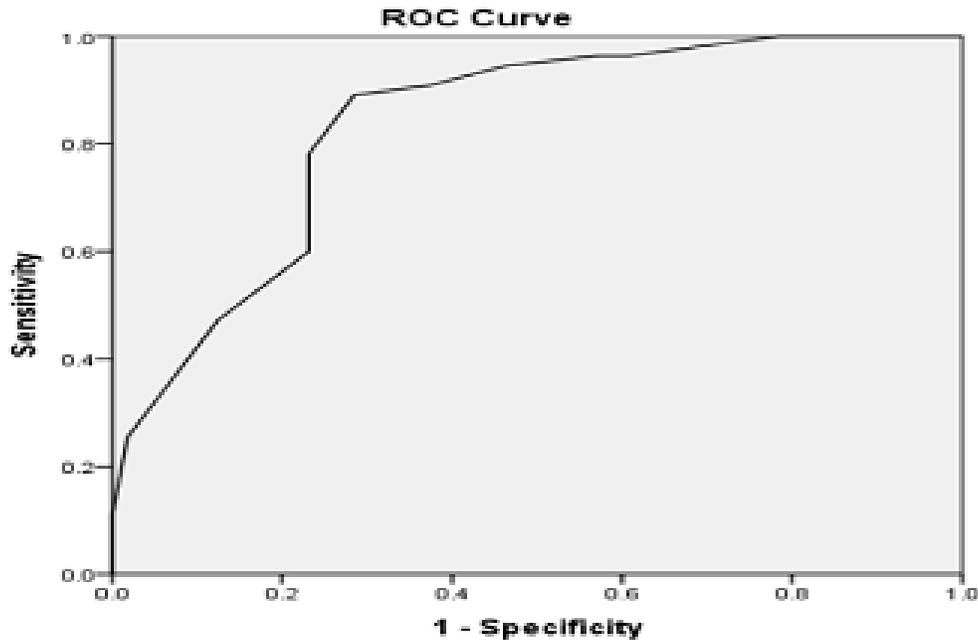
Significant association was seen between mortality and <10 mg/dl HDL level on day 1. Pearson Chi-Square =30.75 with 1dof, P<0.001.

Relative risk of death in patients with day 1 HDL value <10mg/dl was 7.01 times higher than patients with day 1 HDL value ≥10mg/dl [95% confidence interval 2.46 -21.81].

Receiver Operator Characteristic curve:

After calculating the relative risk of death between two groups (HDL<10 mg/dl and HDL≥10 mg/dl) as above, we tried to estimate a single value of HDL on day of admission that can predict overall mortality with reasonable sensitivity and specificity by Receiver Operator Characteristic curve.

Figure 13: Receiver Operator Characteristic curve



The area under the Receiver Operator Characteristic curve for HDL cholesterol was 0.83 (95% confidence interval 0.75–0.90) with standard error 0.03.

A HDL cholesterol cut off value of 9 mg/dl at admission has a sensitivity and specificity of 89% and 73% respectively for predicting the overall mortality.

HDL on day of admission and APACHE 2 score:

As we use mortality scores for calculating the risk of mortality at admission to ICU, we compared day of admission HDL with APACHE 2 to find any correlation.

Table 5: Correlation of Day 1HDL with APACHE 2 score at admission.

SURVIVAL		DOAHDL
Survivors	APACHE2	-.450
Non survivors	APACHE 2	-.163

Current study shows that, among non survivors APACHE 2 score showed significant negative correlation with admission HDL values (pearson correlation -0.163), meaning as the APACHE 2 score is increasing the admission HDL cholesterol was decreasing, both implying increased chance of mortality. Similarly in patients survived, as APACHE 2 score decreased

there was increasing trend in admission HDL value, which translates into low risk of mortality.

Trend of HDL cholesterol:

HDL is an acute phase reactant, we compared day of admission HDL with day 5 HDL, to assess for trend in survivors and non survivors.

Table 6: Comparison of day of admission HDL and day 5 HDL cholesterol with outcome:

DIED		DOAHDL	D5HDL
NO	Mean	11.79	18.34
	N	56	56
	Std. Deviation	5.714	7.681
YES	Mean	5.76	4.91
	N	55	22
	Std. Deviation	2.937	2.308
Total	Mean	8.80	14.55
	N	111	78
	Std. Deviation	5.450	8.977

Above table shows that among survivors, the mean value of HDL on day 5 was increased compared to mean HDL on day of admission (18.34 versus 11.79 respectively), but among non survivors the mean HDL on day 5 was lower than that seen on the day of admission (4.91 versus 5.76 respectively). So, among survivors, rising trend of HDL was observed in our study.

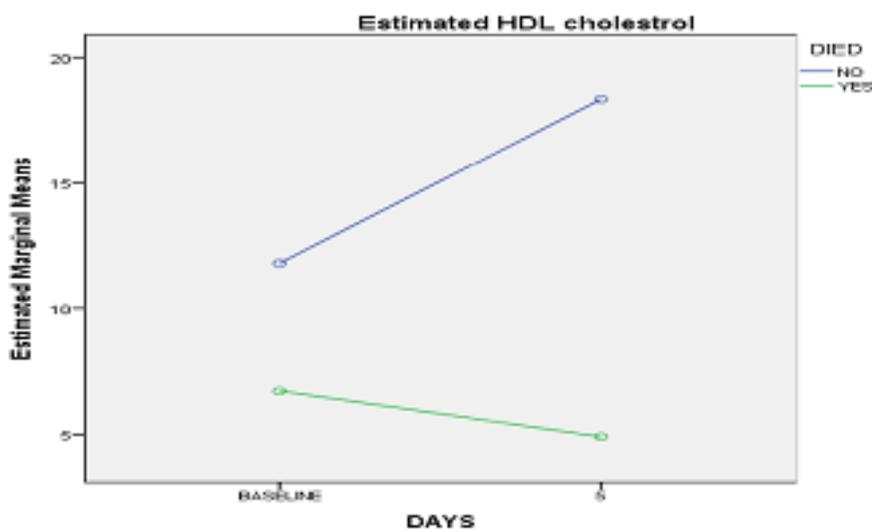
This can be explained logically, as with clinical improvement the burden of bacterial endotoxin in patient's decreases. So less of HDL will be used for neutralization and

decrease in levels of cytokines also may play a role resulting in improvement in serum HDL levels.

Similarly among non survivors, decreasing trend of HDL was seen, implies that as the clinical condition worsens there will be uncontrolled increase in burden of bacterial endotoxin that uses up more HDL for neutralization and persistently raised cytokine levels that suppress HDL production, so the lower levels persisted.

The same trend of HDL among survivors and non survivors is shown below graphically.

Figure 14 shows HDL as negative acute phase reactant.

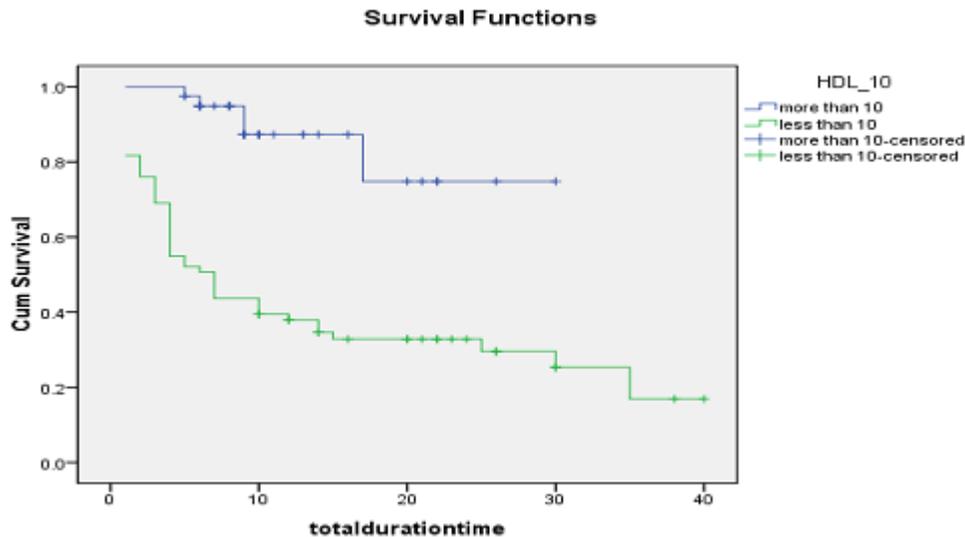


REPEATED MEASURES ANOVA F (df1, df2), 109.961(1, 76), p<0.001 this graph shows, days of illness on X-axis and

mean HDL value on Y- axis. Upper curve for survivors shows that, as the days progressed the mean HDL value was rising and lower curve for non survivors shows decreasing value with time.

Survival analysis: After studying that the relative risk of death, which was higher in patients with day of admission HDL <10 mg/dl compared to ≥ 10 mg/dl, we further studied survival analysis between these groups as showed below in figure 15.

Figure 15: Survival analysis - Kaplan Meier curves.



The above figure shows two curves, Upper curve is for HDL ≥ 10 mg/dl with only 5 deaths out of 40 patients and better survival, lower curve is for patients with HDL <10mg/dl with more number of deaths and prolonged duration of hospital stay.

After showing the association between level of HDL on day of admission and mortality, we studied whether HDL level of day of admission also varied among single organ dysfunction versus multi organ dysfunction.

Association of day 1 HDL with organ dysfunction:

Multi organ dysfunction may be any combination of acute lung injury, acute kidney injury, hypotension, thrombocytopenia and acute liver injury.

Table 7: Association of Organ dysfunction with day1HDL levels.

	Organ dysfunction	Number of patients	Mean HDL on day1	Std. Deviation	Std. Error Mean
DOAHDL	single	33	11.45	5.815	1.012
	2 and more	78	7.68	4.908	.556

Difference in mean HDL value on day of admission was noted between patients presenting with single and multi-organ dysfunction, but p value was not statistically significant. (p<0.186)

IV. DISCUSSION

- Sepsis is one of the leading cause of mortality worldwide.
- In spite of the availability of biomarkers for sepsis and mortality scores, the mortality for sepsis still has not decreased significantly. So, there is need for development of more sensitive biomarkers for early detection of sepsis.

- Lipoproteins are an important part of innate, non-adaptive immune response to infection. (7) HDL-C is the major plasma lipoprotein (15), present in higher concentrations than all other lipoproteins combined.
- To calculate APACHE 2 score we need many parameters like hemodynamic variables which can be readily assessed and laboratory values like haematocrit, WBC count, serum creatinine, acute renal failure, urine output, serum BUN, serum

sodium, serum albumin, serum bilirubin, serum glucose and arterial blood gas analysis many of which may not be available at peripheral community care hospitals. So, measurement of a single value of HDL may be more useful in such setting.

- Any marker which is a part of innate immunity will serve better as an early marker as it has to be breached before full blown sepsis occurs.
- As lipoproteins are an important component of innate immunity and HDL was the main component of lipoproteins highest in concentration in serum and has highest affinity to bind against bacterial endotoxin (LPS in gram negative bacteria and LTA in gram positive bacteria), we studied the role of HDL cholesterol in patients with severe sepsis in medical ICU at a tertiary care hospital as a prognostic marker.

Baseline characteristics:

- Current observational study included 111 patients with varied disease presentations admitted to ICU with severe sepsis after fulfilling the inclusion criteria and exclusion criteria.
- Maximum number of people, 49 out of 111 (44.1%) were in the age group of 41-60 yrs and, 67(60.4%) were males and 44(39.6%) were female.
- All patient's baseline characteristics were comparable. Only high APACHE 2 score and presence of hypotension at admission were more frequently associated with mortality and was statistically significant between survivors and non survivors.
- Others parameters like presence of acute lung injury, acute kidney injury, acute liver Injury (raised bilirubin levels and coagulopathy), baseline serum albumin levels and frequency of dialysis used as renal replacement therapy were comparable in numbers among survivors and non survivors and not found to have effect on mortality to reach statistical significance.
- Number of patients with culture positive infection (blood culture and sputum/ET aspirate culture) (14 in survivors and 17 in non survivors) and positive serology (17 in survivors and 15 in non survivors) were also comparable among two groups.
- Mean HDL on day of admission among culture positive infection patients was 7 mg/dl compared to 9 mg/dl in patients with culture negativity, statistically not significant.
- Similarly difference in mean HDL value among culture positive infection patients didn't reach statistical significance among survivors and non survivors also. So, culture positive infection didn't show statistically significant effect on mortality in current study.

Low HDL at admission and association with mortality in severe sepsis:

- The reason for the low HDL cholesterol value in sepsis is multifactorial, but most important of all is the neutralization of LPS and thus preventing its immune activation and cytokine response.
- Among all lipoproteins, HDL -C has the highest affinity for LPS of gram negative bacteria (16) and LTA of gram positive bacteria (17).
- Studies on plasma HDL-C protecting against bacterial endotoxin dates back to 1993 when Levine DM et al showed that transgenic mice with high HDL-C had high levels of endotoxin bound to HDL-C, low levels of cytokine response and improved survival compared with mice having low HDL-C levels.(6)
- Parker ts et al showed that r-HDL (recombinant HDL) is a potent inhibitor of the induction of TNF-alpha by LPS from gram-negative bacteria in whole human blood. (18)
- Low admission HDL cholesterol levels in patients with sepsis were found in studies conducted by Gordon et al (19) and Shor R et al.(20) Gordon et al study comprised of 32 patients divided into two groups, 12 patients with infection and 20 patients without infection in surgical ICU. The mean HDL value was significantly lower in infected group (19 mg/dl) compared to non-infected group (28 mg/dl). Among infected patients, the mean HDL among survivors and non survivors was not found to be statistically significant, probably because of small size of the sample studied.(65)
- Shor R et al (20) conducted retrospective analysis of patients admitted at Edith Wolfson Medical Centre during a period of 1 year (January 1st 2005-December 31st 2005) and analysed data. They divided patients into two groups, group 1 with HDL \leq 20 mg/dl (108 patients) and group 2 with HDL \geq 65 mg/dl (96 patients). Out of 204 patients 32 had sepsis and all were in group 1, showed association of sepsis with low HDL level. Similarly 42 patients died, of them low HDL was observed in 42.6% of patients who survived vs. 92.9% of those who died ($P < 0.0001$). By using logistic regression analysis, they showed low HDL (\leq 20 mg/dl) was associated with a 17.5-fold increase in odds for death compared to HDL \geq 65 mg/dl (OR 17.5 95% CI, 5.2-59.04, $P < 0.0001$). Current study noted that there was statistically significant difference in mean admission day HDL cholesterol between survivors (5.76 mg/dl) and non survivors (11.79 mg/dl), $p < 0.001$.

Relative risk of death:

- Shor R et al(20) retrospective analysis of 204 patients showed low HDL (\leq 20 mg/dl) was associated with a 17.5-fold increase in odds for

death compared to HDL ≥ 65 mg/dl (OR 17.5 95% CI, 5.2–59.04, $P < 0.0001$).

- Current prospective study showed that low HDL at admission ≤ 10 mg/dl was associated with a 7.01 fold increase in odds for death compared to HDL ≥ 11 mg/dl [95% confidence interval 2.46 - 21.81, $P < 0.001$].
- The difference in relative risk observed in current study compared with Shor R et al was because of the difference in HDL values of the two groups compared (< 10 mg/dl and ≥ 10 mg/dl in current study whereas HDL ≤ 20 mg/dl and ≥ 65 mg/dl by Shor R et al (20)).

Receiver Operator Characteristic curve:

- With the above data showing the relative risk of death between two groups (HDL < 10 mg/dl and HDL ≥ 10 mg/dl), we further studied HDL as a continuous variable and tried to detect a single HDL value with which we can predict mortality with reasonable sensitivity and specificity by receiver operator characteristic curve.
- The area under the receiver operator characteristic curve for HDL cholesterol was 0.83 (95% confidence interval 0.75–0.90) with standard error 0.03. A HDL cholesterol cut off value of 9 mg/dl at admission has a sensitivity and specificity of 89% and 73% respectively for predicting the overall mortality.
- On comparison, study by Chien JY et al (21), showed HDL ≤ 20 mg/dl had a sensitivity of 92%, a specificity of 80% in predicting overall mortality. Cut off value for Day 1 HDL in the current study was lower than that observed by Chien JY et al.
- Lower sample size (63 in Chien JY et al compared to 111 in current study), and difference in the baseline HDL values provides the best explanation. Baseline HDL values were different among Indian population studied in current study from Chinese population studied by Chien JY et al.
- Studies on baseline HDL values showed that Indian population have lower value as shown in study by Sawant AM et al (22) compared to higher baseline HDL value of Chinese population showed in study by Cai HJ et al. (23)

HDL on day of admission and APACHE 2 score:

- In current study we compared HDL on day 1 value with APACHE 2 score for prediction of mortality.
- Pearson correlation analysis showed significant negative correlation (-0.163 Pearson coefficient) between HDL at admission with APACHE 2 score on admission in non survivors, correlation was more with survivors (-0.450 Pearson coefficient).
- Patient with low initial HDL value had higher APACHE 2 score which implies high risk of mortality, as the p value was significant.

Trend of HDL cholesterol:

- Van Leeuwen HJ et al (7) and Dunham CM et al (24) observed finding of rapidly changing HDL levels in severe sepsis patients, but statistical association with mortality was not found, probably because of smaller sample size (17 and 28 patients respectively) compared to the current study with a small sample size of 111.
- Dunham CM et al (24) study involved 28 patients, upon admission to the surgical intensive care unit the mean total cholesterol level was only 119 ± 44 mg/dl, as compared with the expected normal cholesterol level (taken from a database) of 201 ± 17 mg/dl ($P < 0.001$). The 25 survivors had higher total cholesterol at SICU discharge (143 ± 35 mg/dl) relative to admission (112 ± 37 mg/dl; $P < 0.0001$). In the three patients who died, the admission cholesterol was 175 ± 62 mg/dl and the cholesterol at death was 117 ± 27 mg/dl. Van Leeuwen HJ et al (20) studied dynamics of plasma lipoproteins in 17 patients with sepsis and observed dynamic nature of HDL cholesterol, they found low HDL value on day 1 (32 mg/dl ± 4), lowest on day 3 (16.2 mg/dl ± 3) and gradually increased up to 4 weeks in survivors, but there was no correlation of HDL value with mortality. They looked at the trend of HDL but comparison of trend among survivors and non survivors was not analysed in the study.
- Both the studies [Van Leeuwen HJ et al (7) and Dunham CM et al (24)] showed HDL acts as acute phase reactant during sepsis (values change dynamically), but though patients had low cholesterol levels in both studies (total cholesterol in Dunham CM et al and HDL in Van Leeuwen HJ et al) statistical difference between survivors and non survivors was not found, probably because of small sample size and heterogeneity of population involved in both the studies.
- Similar to above studies, current study demonstrated that HDL-C acts as negative acute phase reactant in sepsis, HDL-C values varied with time in both survivors and non survivors. There was increase in HDL-C value, as patient was improving in survivor group but HDL-C remained same or decreased in non-survivors group.
- But current study showed the difference with time among survivors and non survivors was statistically significant. ($p < 0.001$, repeated measures ANOVA).

Survival analysis:

- Kaplan-Meier estimates of survival in patients of severe sepsis showed significant difference in survival between two groups (HDL < 10 mg/dl and HDL ≥ 10 mg/dl group).
- Group with HDL ≥ 10 mg /dl had significantly improved chance of survival compared to group HDL < 10 mg/dl shown in figure 15 by the gap between the groups. The plot also shows the less

number of events (death) in group with HDL \geq 10 mg/dl and also they were discharged more early than patients in HDL <10 mg/dl group.

- Our results were similar to study done by Chien JY et al where survival analysis was done between two groups HDL>20 mg/dl and HDL<20 mg/dl.(21)

Day of admission HDL with organ dysfunction:

- Difference in mean HDL value was noted between patients with single organ failure and multi organ failure but it was not statistically significant in the current study. (p <0.186)
- Multi organ dysfunction in current study may be any combination of acute lung injury, acute kidney injury, hypotension, thrombocytopenia and acute liver injury.

V. CONCLUSION

- Most common cause for origin of sepsis was acute lung injury, found in 89 patients (80.18%), seventy eight patients (70.2%) had atleast two organ dysfunction. Septic shock was present in 69 patients (62.16%)
- Gram negative organisms were isolated in 30% of patients commonest being Escherichia coli, whereas gram positive were found in only 8% of patients with commonest being Enterococcus.
- Number of patients with culture positive infection (blood culture and sputum/ET aspirate culture together) (20 in survivors and 22 in non survivors) and positive serology (21 in survivors and 18 in non survivors) were also comparable among two groups.
- Mean HDL on day of admission among culture positive infection patients was 7 mg/dl compared to 9 mg/dl in patients with culture negativity, statistically not significant. Similarly difference in mean HDL value between survivors and non survivors among culture positive infection patients didn't reach statistical significance. So, culture positive infection didn't have statistically significant effect on mortality in current study.
- There is significant association of low HDL value on day 1 with mortality.
- HDL value of 9 mg/dl at admission had sensitivity of 89% and specificity of 73% predicting the overall mortality in patients with severe sepsis.
- Risk of death in patients with day 1 HDL value <10mg/dl is 7.01 times the risk of death in patients with day 1 HDL value \geq 10mg/dl [95% confidence interval 2.46 -21.81].
- Trend of HDL correlated with clinical outcome of patients. Raising trend favours improvement in clinical condition and decreasing trend implied worsening of the clinical condition.
- Baseline HDL value correlated with APACHE 2 score in predicting mortality in ICU Patients.

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