ABSTRACT

Background: Delay in diagnosis and initiation of antibiotics treatment during sepsis have been shown to increase mortality. Biomarkers can play an important role in diagnosis and prognosis of sepsis. We aimed to evaluate the correlation between septicemia and high density lipoproteins (HDL) level in burned patients. Method: A prospective study conducted at Al-Sadr teaching hospital, Maysan, Iraq, during period from April to September 2013. Blood samples were collected from patient every other day to measure the level of HDL and triglycerides. Other blood samples were collected in blood culture tubes for culturing to verify septicemia. Results: Seventy five patients were admitted consecutively into burn unit, 35 of them (46%) developed septicemia. About 11 patients of the 35 patients are died. All died patients had HDL value (< 5 mg/dl) 1 or 2 days before death since our blood samples were collected every 2 days. Patients with high density lipoproteins (HDL) value < 15 mg/dl were with high risk of developing sepsis. Conclusion: There was a strong correlation between HDL level and septicemia in burned patient. HDL value is a good biomarker for sepsis, it decreases below normal level and continues to diminish and reach to immeasurable level at advance stage of septicemia. Word account (215) Tables (5) Figures (3)

Key words: antibiotic, biomarkers, burns high density lipoproteins (HDL), septicemia, and triglycerides

INTRODUCTION

Sepsis is a leading cause of death in critically ill patients despite the use of modern antibiotics and resuscitation therapies. [1] The septic response is an extremely complex chain of events
involving inflammatory and anti-inflammatory processes, humoral and cellular reactions and circulatory abnormalities. [2, 3] The diagnosis of sepsis and evaluation of its severity is complicated by the highly variable and non-specific nature of the signs and symptoms of sepsis. [4] However, the early diagnosis and stratification of the severity of sepsis is very important, increasing the possibility of starting timely and specific treatment. [5, 6]

Biomarkers can have an important place in this process because they can indicate the presence or absence or severity of sepsis, [7, and 8] and can differentiate bacterial from viral and fungal infection, and systemic sepsis from local infection. Other potential uses of biomarkers include roles in prognostication, guiding antibiotic therapy, evaluating the response to therapy and recovery from sepsis, differentiating Gram-positive from Gram-negative microorganisms as the cause of sepsis, predicting sepsis complications and the development of organ dysfunction (heart, kidneys, liver or multiple organ dysfunction). However, the exact role of biomarkers in the management of septic patients remains undefined. [9] C-reactive protein (CRP) has been used for many years [10, 11] but its specificity has been challenged. [12] Procalcitonin (PCT) has been proposed as a more specific [13] and better prognostic [14] marker than CRP, although its value has also been challenged. [15] It remains difficult to differentiate sepsis from other non-infectious causes of systemic inflammatory response syndrome, [16] and there is a continuous search for better biomarkers of sepsis.

Recent studies suggested that HDL has potent bactericidal and bacteriostatic effects against bacteria [17]. In addition it has a protection activity against the adverse consequences of bacterial infection by neutralizing endotoxins of bacteria. This toxin-neutralizing activity has been shown to be effective against Lipopolysaccharide (LPS) and lipoteichoic acid (LTA) of gram positive bacteria (18).

This study has been shown that all patients with HDL-cholesterol less than 15 mg/dl were with positive blood culture even in the absence of inflammatory reaction (sepsis). This fact indicates that HDL-C level can be used as a biomarker for the early prediction of septicemia. On the other hand, all patients with septicemia (blood infection with systemic inflammation) were with HDL-C level less than 5 mg/dl. These facts suggest that HDL-cholesterol play an important role in blood defense mechanism during microbial infection, or it may suggest that while HDL level was more than 5 mg/dl, sepsis will not be happen.
Other recent studies demonstrated that HDL plays an important role in immunity and during infections and sepsis. [19] These findings suggest that HDL protect against inflammation. However, chronic inflammation modifies HDL from a molecule with anti-inflammatory properties to one with proinflammatory properties, which leads to complex interpretation of plasma HDL-C levels. Although recent genetic and proteomic studies have unveiled important molecular players in HDL metabolism and immune activity, the mechanism for HDL regulation by these molecules remains unclear. [20]

**METHOD AND MATERIALS**

A prospective study, conducted at 20-bed burn unit of Al-Sadr teaching hospital, Maysan, Iraq, during period from April to September 2013 to evaluate the correlation between HDL level and sepsis.

The study protocol was designed as per recommendations of the Standards for Reporting of Diagnostic Accuracy steering committee. [21]

During our study, 75 patients were admitted into burn unit. Patients died in the first 48 hours, patient on antihyperlipidemic agents (statins) and patients referred into another unit or centers were excluded from this study.

Two tubes of blood samples were collected from patients every other day (every2 days). One for biochemistry tests: (lipid profile, blood urea, serum creatinine, liver profile, full electrolyte profile) and another for complete blood count (CBC).

The blood samples for biochemical tests were subjected to centrifugation for 5 minutes at 4000 rpm to extract serums which then pipette into special tubes of ARCHITECT® c 4000 system, which is fully automated system to measure lipid, urine, liver and full electrolytes profiles. These results then printed in one report with the normal range of each test. Another blood samples were collected in blood culture tubes of VITEK 2 system, every 5 days to verify the growth of bacteria (septicemia). Wound swabs were taken from 3 different sites every 3 days to verify wound infection. Wound site, percentage of total burn surface area (%TBSA), degree, depth, presence or absence of slough and source of burn injury were clinically assessed. Patients were stabilized by fluid resuscitation (Ringer’s IV fluid) with analgesic and wound management with silver sulfadiazine and dressing after cleaning and
debridement. Antibiotics were initiated according to the results of bacteriological examinations. This study was approved by the ethical committee of our hospital.

RESULTS

Ninety one patients had been admitted into our burn unit from April through September 2013. 16 patients excluded from our study for the following reasons: patients died during first 48 hours of admission (No. 9), patients with atherosclerosis or were on antihyperlipidemic medicines (statins), (No. 4). Patients referred into other units or centers due to different reasons (No. 3).

After exclusion, 75 patients were enrolled in this study. Patients were with different burns size (%TBSA) average 33.5% with range (15%- 95%). 61% of patients were female and 39% were male. 45% of burns were scalds, 49% were with direct flame and 6% with electricity. Patients’ age range from 1- 85 years with average was 17 years (table: 1)

Table 1 Patient’s characteristics; age, sex, %TBSA and burn types.

<table>
<thead>
<tr>
<th></th>
<th>Average</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>17</td>
<td>(1- 85)</td>
</tr>
<tr>
<td>%TBSA</td>
<td>33.5%</td>
<td>(: 15%- 95%)</td>
</tr>
<tr>
<td>Sex</td>
<td>Female 61%</td>
<td>Male (39%)</td>
</tr>
<tr>
<td>Burn types</td>
<td>Scalds : 45% Flame : 49% Electricity : 6%</td>
<td></td>
</tr>
</tbody>
</table>

Lipid profiles of patients during onset of burns injuries were: HDL level (30-56 mg/dl) with average level 39 mg/dl, triglycerides (37-148 mg/dl) with average level 70 mg/dl, and cholesterol levels were (46-155 mg/dl) with average level 78 mg/dl. Blood cultures and wound swabs were negative (no growth of bacteria) table: 2.

Table 2 lipid profile for all 75 patients at the onset of burns injury with normal range.

<table>
<thead>
<tr>
<th>Lipid profile</th>
<th>Range (mg/dl)</th>
<th>Average( mg/dl)</th>
<th>Mode( mg/dl)</th>
<th>Normal range (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High density lipoproteins (HDL)</td>
<td>30- 56</td>
<td>39</td>
<td>38</td>
<td>39-59</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>37- 148</td>
<td>70</td>
<td>58</td>
<td>0-149</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>46- 155</td>
<td>78</td>
<td>86</td>
<td>0- 199</td>
</tr>
</tbody>
</table>
From 75 patients there were 35 (46.7%) patients complaining of septicemia during our study. There was severe dropping in high density lipoproteins (HDL) level during septicemia; blood culture was positive, in addition to systemic inflammatory response syndrome (SIRS) which included hypo- or hyperthermia, tachycardia, tachypnea and WBC count was out of normal range (4000-12000/uL).

Table 3 lipid profile at the onset of sepsis.

<table>
<thead>
<tr>
<th>Lipid profile</th>
<th>Range (mg/dl)</th>
<th>Average (mg/dl)</th>
<th>Mode (mg/dl)</th>
<th>Normal range (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High density lipoproteins (HDL)</td>
<td>4-13</td>
<td>7.6</td>
<td>4</td>
<td>39-59</td>
</tr>
<tr>
<td>triglycerides</td>
<td>133-435</td>
<td>214.5</td>
<td>180</td>
<td>0-149</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>56-139</td>
<td>82.8</td>
<td>76</td>
<td>0-199</td>
</tr>
</tbody>
</table>

There were significant differences in level of high density lipoproteins during onset of burns and sepsis (p<0.01), also in level of triglycerides (p<0.01), while there was no significant difference in level of cholesterol. (Table: 4)

Table 4 change in lipid profile at burns and sepsis onset with p-value.

<table>
<thead>
<tr>
<th></th>
<th>HDL</th>
<th>triglycerides</th>
<th>cholesterol</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burn onset</td>
<td>39</td>
<td>70</td>
<td>78</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Sepsis onset</td>
<td>7.6</td>
<td>214.5</td>
<td>76</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

During this study about 68.5% of patients with sepsis were thrombocytopenic (average 121000/uL), and all of them were with low serum albumin (average: 2g/dl), while serum creatinine and blood urea were within normal level (average: 0.51 and 12mg/dl) respectively for all patients (table: 5)

Table 5 levels of urea, creatinine, albumin, WBC, platelets and time from onset of burns to onset of sepsis.

<table>
<thead>
<tr>
<th></th>
<th>%TBSA</th>
<th>WBC(*1000/ul)</th>
<th>Platelets count(*1000/ul)</th>
<th>Blood urea (mg/dl)</th>
<th>Serum creatinine</th>
<th>Serum albumin (g/dl)</th>
<th>Time to get sepsis (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>55%</td>
<td>11.86</td>
<td>154.9</td>
<td>14</td>
<td>0.51</td>
<td>2.0</td>
<td>7</td>
</tr>
<tr>
<td>Mode</td>
<td>45%</td>
<td>9.04</td>
<td>121</td>
<td>12</td>
<td>0.37</td>
<td>1.8</td>
<td>10</td>
</tr>
<tr>
<td>Min</td>
<td>27%</td>
<td>2.27</td>
<td>32</td>
<td>9</td>
<td>0.32</td>
<td>1.4</td>
<td>2</td>
</tr>
<tr>
<td>Max</td>
<td>95%</td>
<td>15.03</td>
<td>535</td>
<td>21</td>
<td>0.73</td>
<td>3.1</td>
<td>20</td>
</tr>
</tbody>
</table>
The area under the curve of Receiver Operating Characteristic (ROC) curve was 0.9922 and 95% confidence limits of area was = (0.9769, 1.000). This indicates the accuracy of diagnosis and sensitivity of HDL level for sepsis (figure 1).

![ROC for Surrogate - HDL, Gold Standard - DIAGNOSIS](image)

**Figure 1. The area under the curve of ROC curve, the specificity was 98%.**

11 patients died complaining of sepsis. During severe sepsis, all patients were with high density lipoproteins (HDL) level less than 5 mg/dl accompanied with hyperglyceridemia while during death were with immeasurable HDL level with normal triglycerides level.

**DISCUSSION**

Biomarkers can play an important role in diagnosis and prognosis of sepsis due to the increase in the mortality rate caused by sepsis, also septicemia considered to be the leading cause of mortality in burn unit. This biomarker; therefore, that the search for a highly accurate biomarker of sepsis has become one of the holy grails of medicine. During our research it is very clear to find a relationship between HDL value and developing of septicemia and severe sepsis.

The effect of microbial infections on HDL level is thought to be through different mechanisms: Many studies suggest that Lipopolysaccharide (LPS) is the primary cause of sepsis induced by gram-negative bacteria. LPS, LPS-binding protein, CD14, and the toll-like receptor 4 (TLR4) complexes induce macrophage activation.[22] HDL, particularly apoA-I, decreases macrophage activation by binding and neutralizing LPS [23] also numerous studies have shown that lipoproteins bind microorganisms when either endotoxin (LPS), from gram negative bacteria, or lipotiechonic acid (LTA), from gram positive bacteria. The majority of the LPS and LTA are bound to HDL. This binding to HDL inhibits the ability of LPS and LTA to interact with toll-like receptors (TLR) and activate macrophage.[24]
Another study suggests that HDLs play a major role in the binding and clearance of circulating endotoxin to the bile and thereby inhibits endotoxin-induced cellular activation, resulting in potent anti-infectious activity.[25] During onset of burns, high density lipoproteins (HDL) level was lower than normal value in most patients in our study especially in large burns size (> 45%); it decreases while burn size increases; HDL level range was (30-56mg/dl) and the %TBSA range was (15%- 95%) see (table: 1) and (table: 2). While triglycerides and cholesterol were within normal range, it is thought to be due to inflammatory process following burns that reduce the concentration of HDL. [26] In comparison with lipid profile during onset of septicemia (figure: 2), there were abnormal level of both high density lipoproteins and triglycerides (table: 3).

![Lipid profile at burns onset and sepsis onset](image)

**Figure 2.** Show the significant differences in lipid profile during burns and septicemia onset.

In this study, all patients with positive blood culture and systemic inflammatory response syndrome were with HDL level less than 15 mg/dl accompanied with hypertriglyceridemia (figure: 3). The average of HDL level during the onset of sepsis was (7.6mg/dl) while the average of triglycerides was (214.5mg/dl) see (table: 3).

![Lipid profile during the onset of septicemia](image)

**Figure 3.** Lipid profile during the onset of septicemia, low HDL level and hypertriglyceridemia.
Hypertriglyceridemia accomplished with low HDL level especially in sepsis was seemed to be due to the destruction of HDL particles and the fact that the HDL core enrichment in triglyceride (60%-80%) of core lipid. Also in non-acute phase conditions in humans, there is a close relationship between hypertriglyceridemia and low HDL.[27] In our study hyperglyceridemia may reach to very high level 435 mg/dl (table: 1). this inverse relationship between plasma HDL and triglyceride levels may be partly due to the ability of HDL to regulate triglyceride metabolism.

**Take home messages**

. HDL value was below normal level in all burnt patients and it continues to decrease until it reaches to < 15 mg/dl when septicemia happened (blood culture positive).

. During septicemia HDL level decreases to immeasurable value accomplish with hyperglyceridemia.

. The levels of both HDL and triglyceride are start again to elevate when sepsis is cured until reach to the normal value.

. HDL value can be used as a biomarker of septicemia, it is easy, cheap and the best septicemia biomarker due to the wide range of HDL normal value (40 – 60 mg/dl) compared with other biomarkers, also wide range from normal value to HDL value in septicemia ( from 40 mg/dl to 15 mg/dl).

**ACKNOWLEDGEMENTS**

We would like to thank the nursing staff of the burn unit, and workers in the laboratory of the al-Sadr teaching hospital for their assistance in collecting and analyzing blood samples.
Also special thanks to Ahmed al-Nuri (pharmacist assistant) for his great support in accomplishing this project.

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