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# Comparison of Complement component C3 level with bacterial infections in decompensated liver disease patients in a tertiary care hospital

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**Abstract**: Bacterial infections are more common in decompensated liver disease patients with low level of complement component 3 and causes 30%-50% of deaths. Therefore, this study done to determine the various bacterial agents causing infections in decompensated liver disease patients and to estimate the level of C3 component of complement by Enzyme linked immunosorbent assay. A prospective study was conducted over a period of one year in Madras Medical College. Ascitic fluid, urine, sputum, wound swab blood and serum were collected. All the samples were processed through Gram's stain and culture. The organisms were identified by standard protocols and antibiotic susceptibility testing and to correlate the bacterial infections with complement C3 level by ELISA. Out of 100 samples, culture positivity seen in 54[54%]. In 54 culture, positive isolates, 41(76%) were Gram Negative bacilli and 13 (24%) were Gram Positive cocci. Among Gram negative bacilli, *Escherichia coli* and in Gram positive cocci, *Staphylococcus aureus* was the most common isolates. The most common infections were spontaneous bacterial peritonitis [29.6%] followed by urinary tract infections [26%], pneumonia [20.3%], Spontaneous bacteraemia [16.7%], and skin and soft-tissue infections [7.4%]. Out of 100patients, 66(66%) patients had low complement component C3 level. Of which 42(63.6%) patients were cultures positive and 24(36.4%) were culture negative. 34(34%) patients had normal complement component C3 level, of which 29 (85.3%) were culture negative and 5 (14.7%) were culture positive.

**Key words**: Complement component C3; Decompensated liver disease; spontaneous bacterial peritonitis; *Escherichia coli; Staphylococcus aureus;* Enzyme linked immunosorbent assay

## Introduction

Liver failure leading to cirrhosis is one of the most common causes of death in our country [1, 2]. Cirrhosis is a chronic progressive liver disorder caused by alcoholic liver diseases, viral hepatitis (HBV and HCV) and cryptogenic causes [3] which can leads to liver failure and death [4]. According to the stages of liver injury, signs and symptoms and survival rate, cirrhosis is classified into compensated and decompensated liver disease. [5].

Decompensated liver disease (DCLD) is defined as irreversible chronic injury of the hepatic parenchyma and extensive fibrosis in association with the formation of regenerative nodules and leading to loss of liver function [6]. Immune dysfunction in the DCLD patients is multi factorial [7, 8]. Impaired function of the reticuloendothelial system, deficiency of complement component level mainly C3, impaired opsonisation activity also decrease in bactericidal activity [9, 10 & 11] have been implicated in the pathogenesis of the increased susceptibility to infections of patients with DCLD.

The phagocytic function of the reticuloendothelial cells are reduced due to intra hepatic shunting of blood in cirrhotic patients [12, 13]. The reduced serum concentration of complement and fibronectin play an important role in the decreased action of reticuloendothelial system (RES) [14]. Kupffer cell is the main component of monocyte macrophage system. Impaired Kupffer cell function in cirrhosis liver leads to significantly reduced phagocytic activity and bactericidal activity [15]. Acquired deficiency of certain complement components especially C3 in serum occur because Complement component C3 is mainly synthesized from hepatocytes of liver and its concentration in ascitic fluid is significantly reduced in patients with advanced cirrhosis [29]. Complement component 3 is one of the important prognostic factors to assess the severity of cirrhosis [16].

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**The following mechanisms contribute to low level of serum complement in DCLD patients**

1. Some complement component are directly synthesized by hepatic parenchymal cells and their synthesis may be reduced as a direct consequence of injury and death of hepatic parenchymal cells [17]
2. Extra hepatic synthesis of other complement components are also reduced due to metabolic disturbance associated with liver failure [18].
3. There may be circulating in activators for complement components are also present.
4. There may be increased consumption of complement by antigen- antibody complex [19].
5. Increased catabolism or increased loss of complement component into the urinary or gastrointestinal tract.

The concentration of the third component of complement (C3) in ascitic fluid and serum appears to have the best predictive value for bacterial infections [20]. In decompensated liver disease patients, the spontaneous bacterial peritonitis (SBP) is a serious common bacterial infection, followed by urinary tract infections (UTI), spontaneous bacteraemia, pneumonia, and skin infections [8]. The common causative organisms for bacterial infections in DCLD patients are *Enterobacteriaceae*, nonfermentable gram-negative bacilli and Gram positive cocci and most of them are multidrug resistant [21-22]. The prognosis of these patients is closely related to a prompt and accurate diagnosis and appropriate treatment decreases the mortality rates.

## Materials and Methods

A prospective study was conducted over a period of one year at the Institute of Microbiology, Madras Medical College, Chennai. About 100 DCLD patients (≥18 yrs), admitted in various wards with signs & symptoms suggestive of bacterial infections are included in the study. Ascitic fluid, urine, sputum, wound swab, blood and serum were collected. All the samples were processed through Gram's stain and inoculated onto Blood agar plate, Chocolate agar and MacConkey agar. The inoculated culture plates were incubated overnight at 37°C in a incubator. A Gram's stain was done the next day from the growth and examined. The organisms were identified by standard protocols and antibiotic susceptibility of recommended drugs (CLSI guidelines) was performed by using Kirby Bauer disc diffusion method. Human Complement component C3 level was detected by Enzyme linked immunosorbent assay in serum by using AssayMax Human Complement C3 ELISA Kit manufactured by ASSAYPRO agencies. The technique of ELISA was performed as per the manufacturer guidelines.

## Results

Out of 100 samples, culture positivity seen in 54[54%]. In 54 culture, positive isolates, 41(76%) were Gram Negative bacilli and 13 (24%) were Gram Positive cocci. Among Gram negative bacilli, *Escherichia coli* and in Gram positive cocci, *Staphylococcus aureus* was the most common isolates. The most common infections were spontaneous bacterial peritonitis [29.6%] followed by urinary tract infections [26%], pneumonia [20.3%], Spontaneous bacteraemia [16.7%], and skin and soft-tissue infections [7.4%] [Table: 1].


**Figure 1**: Human Complement C3 Elisa Kit

**Table 1:** Samples distribution and bacterial infections in DCLD patients (n = 100)

| S. No | Type of Sample | Number of Sample (%) (N=100) | Culture Positive (%) (N=54) |
|---|---|---|---|
| 1 | Ascitic fluid –Spontaneous bacterial peritonitis | 42 (42%) | 16 (29.6%) |
| 2 | Urine- UTI | 27 (27%) | 14 (26%) |
| 3 | Sputum- Pneumonia | 21 (21%) | 11 (20.3%) |
| 4 | Wound swab- Skin infections | 10 (10%) | 4 (7.4%) |
| 5 | Blood- Spontaneous bacteraemia | 100 (100%) | 9 (16.7%) |

**Table 2:** Organisms isolated

| Organisms (no=54) | Ascitic fluid | urine | Sputum | Skin | Blood | No | % |
|---|---|---|---|---|---|---|---|
| *Escherichia coli* | 5 | 6 | 0 | 1 | 2 | 14 | 25.9 |
| *Klebsiella pneumoniae* | 2 | 2 | 4 | - | 1 | 9 | 16.65 |
| *Klebsiella oxytoca* | 1 | 3 | 4 | - | - | 8 | 14.8 |
| *Proteus vulgaris* | - | - | - | 1 | - | 1 | 1.85 |
| *Enterobacter cloacae* | 1 | 1 | - | - | - | 2 | 3.7 |
| *Citrobacter koseri* | 1 | - | 1 | - | - | 2 | 3.7 |
| *Pseudomonas aeruginosa* | 1 | - | 1 | - | - | 2 | 3.7 |
| *Acinetobacter baumanii* | 1 | 1 | 1 | - | - | 3 | 5.55 |
| *Staphylococcus aureus* | 2 | - | - | 2 | 2 | 6 | 11.2 |
| *Enterococcus faecalis* | 1 | 1 | - | - | 1 | 3 | 5.55 |
| *Staphylococcus epidermidis* | - | - | - | - | 2 | 2 | 3.7 |
| *Streptococcus viridians* | 1 | - | - | - | 1 | 2 | 3.7 |
| Total | 16 | 14 | 11 | 4 | 9 | 54 | 100 |

Most of the organisms were 75% sensitive to amino glycosides and 50% sensitive to fluoroquinolones. All the GNB were 100% sensitive to carbapenem.

**Table 3:** Detection of complement component C3 concentration by ELISA

| C3 value | Total sample (n=100) | Culture Result | |
|---|---|---|---|
| | | Positive | Negative |
| Value less than 0.4mg/ml | 66 (66%) | 42 (63.6%) | 24 (36.4%) |
| Value more than0.4mg/ml | 34 (34%) | 5 (14.7%) | 29 (85.3%) |

Out of 100patients, 66(66%) patients had low complement component C3 level. Of which 42(63.6%) patients were cultures positive and 24(36.4%) were culture negative. 34(34%) patients had normal complement component C3 level, of which 29 (85.3%) were culture negative and 5 (14.7%) were culture positive. (Table 3)

## Discussion

Among 100 DCLD patients, males 97 (97%) were predominant group when compared to females 3(3%). This predilection of higher frequency rates among male is attributed towards the presence of underlying risk factors like alcoholism [23-24].

Of the 100 samples, culture positivity seen in 54[54%] In 54 culture positive isolates, 41(76%) were Gram Negative bacilli and 13 (24%) were Gram Positive cocci. Among Gram negative bacilli, *Escherichia coli* was the most common isolates and in Gram positive cocci, *Staphylococcus aureus* was the most common isolates which was correlated significantly [P value = 0.005]. In decompensated liver disease (DCLD) patients, the most common isolates were Gram negative bacilli which may be due to translocation of normal flora (most of the normal flora in the GIT are GNB) from the gastro intestinal tract. Among bacterial infections, *Escherichia coli* were the most common pathogen (25.9%) [25].

Among culture positive infections, spontaneous bacterial peritonitis (29.6%) was the most common infection due to translocation of enteric organisms from the intestine to the peritoneum and procedures like diagnostic and therapeutic paracentesis. Urinary tract infection (26%) was the second most common infection because of indwelling urinary catheters, followed by pneumonia (20.3%) due to tracheal intubation, oesophageal tamponade, hepatic encephalopathy and alcoholism etc.

Spontaneous bacteraemia (16.7%) because of Porto systemic shunt circulation in DCLD patients will favor the multiplication of organisms and to escape from phagocytosis by hepatic reticuloendothelial system [16], followed skin and soft tissues infection (7.4%) due to lymphangitis of the lower extremities and abdominal wall [23, 26 27, 16, and 28].

Most of the organisms were 75% sensitive to amino glycosides and 50% sensitive to fluoroquinolones. All the GNB were 100% sensitive to carbapenem. In culture positive infections, 51.8% (28/54) drug resistant bacterial infections were identified, mainly ESBL followed by MRSA, and VRE.

Out of 100patients, 66(66%) patients had low complement component C3 level. Of which 42(63.6%) patients were cultures positive and 24(36.4%) were culture negative. 34(34%) patients had normal complement component C3 level, of which 29 (85.3%) were culture negative and 5 (14.7%) were culture positive. Complement component C3 concentration level in serum were decreased in decompensated cirrhotic patients with infections compared with decompensated cirrhotic patients without infections. Concentrations of complement component C3 level significantly correlated (p=0.001) with decompensated liver disease (DCLD) patients with infections. Many

studies conducted by Mustafa G *et al.,* 2007 [29], Alper, C.A. *et al.,* [17] and Colten H.R *et al.,* 1972[18] reported that the complement is mainly synthesized by hepatocytes of liver. Due to cirrhosis, all the hepatocytes are destroyed and unable to synthesize complement component C3. This reduced level of complement C3 is the one of the risk factors for bacterial infections in DCLD patients [30, 31].

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## Conclusion

Bacterial infections in decompensated liver disease patients are due to invasive practical procedures, malnutrition, derangement of gut flora leads to intestinal stasis, bacterial over growth, increased intestinal permeability and impaired host defense mechanisms against infection. The complement C3 is mainly synthesized by hepatocytes of liver. Due to cirrhosis, all the hepatocytes are destroyed and unable to synthesize complement component C3. This reduced level of complement C3 is the one of the risk factors for bacterial infections in DCLD patients. Complement component C3 level is one of the important indirect prognostic factors to assess the infection in DCLD patients.

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