Immune Alterations in Liver Cirrhosis: Its Relationship with Etiology, Child Pugh Stage and Malnutrition

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Abstract

Background: Immune alterations in liver cirrhosis are variable and only a few have been correlated with the severity of the disease, malnutrition or its etiology.

Methods: A descriptive transversal study was carried out in 76 patients with liver cirrhosis predominantly of viral cause. According to the Child Pugh stage they were classified in A: 52, B:17 and C: 7. The anthropometric evaluation included mid-arm circumference, triceps and subscapular skinfold thickness. The humoral immune alterations were evaluated by assessing serum immunoglobulin (A, M, G and E) and the complement components’ C3 and C4. Cellular immunity integrated a total lymphocyte count and the delayed intradermal hypersensitivity test. The statistical analysis included Pearson’s Chi squared and non-parametric tests using the U Mann Whitney or Kruskal Wallis test.

Results: In the study group, the immunoglobulins were in normal range; however the complement components C3 and C4 showed a certain tendency to the inferior normal limit. Immunodeficiency was diagnosed in 28 patients (36.8%). Statistical comparisons showed that C4 complement component was the principal factor affected with the lower values in alcoholic and viral group, whereas for the alcohol group, IgE was found at higher titles. The major immunological dysfunction was in Child C stage, found it in 71.4%. Malnutrition was present in 63.2%, but the analysis of the humoral immunity indicators according to the nutritional state only reported significant differences in the C4 average values. A higher percentage of immunodepressed in the undernourished was reflected, in comparison to the non-malnourished.

Conclusion: In liver cirrhosis, the major contribution to the distorted immune response is the Child Pugh stage while the malnutrition maybe has a possible influence.

Keywords: Liver Cirrhosis; Child Pugh; Immunodeficiency; Malnutrition

Introduction

The immune alterations in patients with liver cirrhosis vary, they are not universal and only a few have been able to be correlated with the severity of the disease or its prognosis. The best characterized alterations correspond to the inefficient bactericide action from the serum, opsonins and complement, altered function of neutrophils and to changes in the activity of the reticuloendothelial system[1].

On the other hand, individuals with cirrhosis are severely affected by malnutrition and are therefore susceptible to complications probably derived from humoral and cellular immunologic deficiencies [2-7]. Energy undernourishment in its diverse forms including the subclinical, compromises the resistance to external agents such as bacteria, viruses, fungus and also chemical agents in a severe way [8]. The recovery of immune functions after a nutritional substitution with supplements is encouraging and is another proof of the relationship between nutrition and immune state [9-13]. Cirrhotic patients have an increased intestinal permeability which enables the passage of endotoxins generated by gram negative bacteria from the intestine to the lymphatic and blood stream. The latter stimulates the liberation of mediators of the inflammatory response and nitric oxide, these being responsible in a great manner for the catabolic state and the hyperdynamic circulation of the disease [14].

The immune alterations and the repercussions on the nutritional state caused by alcohol in cirrhotic patients are known, however, investigations that evaluate this association in relation to other causes of cirrhosis like the viral and metabolic have been poorly explored [6].

Although the physiopathologic bases explain the relationship between undernourishment and immune alterations, the results obtained in the different studies related to cirrhosis of the liver are still insufficient mainly because the precise mechanism of immunocompetence in the disease is not well established. The present study was carried out to identify the main immune alterations in patients with cirrhosis of the liver in relationship with the stage of the disease, undernourishment and its etiology.

Material and Methods

A descriptive transversal study was carried out at the Institute of Gastroenterology in Havana, Cuba from March 2008 to May 2010 in 76 patients with a confirmed diagnosis of liver cirrhosis (clinical, laboratory tests and histomorphologic), divided into three groups according to the etiology, 55 viral (34 by hepatitis C virus, 17 by hepatitis B virus and 4 co-infected by both viruses), 11 alcoholics and 10 of a predominantly metabolic cause. A similar distribution of male/female (38/38) was presented with an average age of 50.1 ±8.8 years, classified according to Child Pugh Stage.
the Child Pugh stage with A: 52, B:17 and C: 7. Although the viral cause predominated in both sexes, alcoholism was more in males. Patients with autoimmune diseases, severe allergies, immunosuppressing diseases, presently taking immunosuppressing/immunomodulation drugs (including steroids) or antivirals in the previous six months, immunodeficiency of any type, human immunodeficiency virus (HIV), pregnancy and uncontrolled chronic diseases were excluded.

The etiology of the liver cirrhosis was defined as viral: when the viral markers for hepatitis B virus (HBV) or hepatitis C virus (HCV) were positive; alcoholic: defined by the daily consumption of more than 80g of ethanol in males and more than 60g in females for more than 10 years and other causes: liver disease due to fat deposits or cryptogenic.

The serologic detection of hepatitis B and C virus and HIV were carried out using a second generation ELISA test (Abott's diagnostic kit).

Liver function tests were done on all patients including (reference values), alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) (<50U/L), total serum bilirubin (<17mmol/L), albumin (35-45g/L), prothrombin time (±3sec/control), cholesterol (<5.2mmol/L), glycaemia (<6.1mmol/L) and creatinine (<129mmol/L). These determinations were carried out with the blood chemistry autoanalyzer HITACHI 902 according to the references of Roche Diagnostics. Hemoglobin (115-140g/L in female and of 120-160g/L in males) and platelet count (150-400 x 10⁹/L) were processed in the hematologic complex ABX Micro 60 and alpha-fetoprotein in the Ultra Microanalysis System (SUMA) (<15 U/L/ml). (Diagnostic kits from Centro de Inmunoensayo, Havana, Cuba).

The anthropometric evaluation included mid-arm circumference (MAC) measured in centimeters, triceps skinfold thickness (TST) measured in millimeters and subcapsular skinfold thickness (SST) in millimeters as has been established by norms. With the first two indicators, the arm muscle area (AMA) and arm fat area (AFA) were calculated. Patients were classified as undernourished or non-undernourished according to the values defined in the references consulted for skinfold thickness and mid-arm circumference in the Cuban population [15-17]. Particularly patients with ages that range between 60 and 65 years, were evaluated according to the recommendations established by the World Health Organization for patients over 60 years [18]. To minimize the intraobserver variation in the anthropometric measurements, the average of three consecutive measurements was taken. A metric measuring tape and well callibred skinfold caliper with a pressure of 10 g/mm² of contact surface (Holtain Ltd, Crymych, UK) was used. Measurements were taken midway between the tip of the acromion and the olecranon process, with the patient standing in a relaxed position. Nutritional evaluation was based on values of MAC, TST, SST, AMA, AFA compared with those of a health reference population [15-18]. The classification of malnutrition was based on the Berdasco [17] studies.

The humoral immune alterations were evaluated by assessing serum immunoglobulin (A, M, G and E) and the complement – components’ C3 and C4. The quantitative measurement of immunoglobulin was carried out by the immunonutribiometic methods (Futura System S.r.l, Italy). Reference values were: total count of immunoglobulin: 8-16 g/L, IgA: 0.70 - 4.00 g/L, IgM: 0.40 - 2.30 g/L, IgG: 7.0 - 16.00 g/L and IgE: 25 - 350 kU/L. The reference values for C3 and C4 were 0.9-1.8 g/L and 0.1-0.4 g/L respectively.

Cellular immunity was evaluated by a total lymphocyte count carried out in the automated counter ABX Micro 60 (range: 1.2 to 3.2 x 10⁹/L) and the delayed intradermal hypersensitivity test with 4 types of antigens: 2 of them composed by a mixture of intestinal bacteria (Shigella, Salmonella, Proteus mirabilis, Proteus vulgaris, Proteus rettgerri, Morganella morgani, Pseudomona, Klebsiella, Escherichia coli, Citrobacter sp, Enterobacter) and respiratory bacteria (coagulase-negative staphylococcus, coagulase-positive staphylococcus, alpha hemolytic Streptococcus, Pseudomonas, Klebsiella pneumoniae, Neumococo pneumoniae, Moraxella catharralis). The other two were the candidin and the tetanic toxoid. The injections were given in the left forearm, and the reading was done 48 hours after the antigen had been administered. The nodules was interpreted according to its diameter in the following way: normal if diameter was between 4 to 6 mm, anergicic hyperergic if 0 - 3mm and hyperergic if equal to or more than 7mm. The hypersensitivity tests enabled the classification of an individual as immunodepressed when two or more intradermic tests had nodules smaller than 4mm.

All patients were asked for a written informed consent and the study received the approval of the ethics investigation committee of the institution.

**Statistical analysis**

Descriptive measures, the mean, deviation standard and median were applied on the quantitative variables and for the qualitative variables, absolute and relative frequency was applied. The analysis applied by contingency tables for qualitative variables was done using Pearson’s Chi squared test. The non-parametric tests were used to analyze the differences in indicators of humoral and cellular immunity of independent groups (groups of patients divided by cause, stage of the disease and nutritional state); the U Mann Whitney or Kruskal Wallis test was applied to denote the differences between two or more than two independent groups respectively. The variance analysis ANOVA was used to compare the anthropometric indicators in the different Child Pugh stages, when necessary, an adjustment with post-hoc Bonferroni test was used. A 5% level of significance was adopted in all the analysis to denote differences as significant. The analysis was carried out using SPSS version 15.0 (lænded Scientific, USA).

**Results**

The majority of cirrhotics were of a viral cause, the hematologic and biochemical tests showed differences only in the behavior of the ALAT and ASAT enzymes which were higher in this group. Table 1.

In the cirrhotic group studied, the immunoglobulins were in normal range, however the complement components C3 and C4 (g/L) showed a certain tendency to the inferior normal limit, 0.96±0.36 and 0.17±0.08 respectively. Immunodeficiency was proved in 28 patients (36.8%), of these, the majority had more than two intradermic tests with anergy or hyperergy, most evident with candidin and the respiratory bacteria. The total lymphocyte count didn’t show alterations with a mean between normal values, 1.7±0.8x10⁹/L.

In the analysis of humoral immunity according to the three groups of causes, only C4 showed significant differences with the lower values in alcoholics and viral. Although alcoholics showed the highest average of IgA values, they didn’t significantly exceed the other two groups. Anergy and hyperergy for candida and bacteria of the respiratory tract were more evident in groups of cirrhotics of viral etiology and of other causes although not significantly. Surprisingly, alcoholics showed an average of normal sized nodules in the three of the four intradermic tests carried out. Table 2.

Similar results were obtained in the total lymphocyte count,
immunologic compromise, the major percentage of immunodepressed were in Child C stage with 71.4% while stages B and A were 52.9% and 28.8% respectively (p=0.034).

The results of the anthropometric evaluation of patients in the study are presented in Table 4. The mean age in men was below that of women. In these, the repercussion of the disease over the body composition was more noticeable, evidenced in the progressive decrease of the relative indicators of adipose and muscular reserve with the worsening of the disease. However, in females, the decrease of MAC and AMA was evident between Child Pugh A and B, both cases with Child C stage were obese patients, which justifies the values of the anthropometric indicators. Overall, 48 undernourished patients were detected (63.2%).

The analysis of the humoral immunity indicators according to the nutritional state, only reported significant differences in the average independent of the etiology of cirrhosis; values above 1.2 X 10^9/l were obtained. The average values for total lymphocyte count for viral, alcoholic and other causes were 1.8 ± 0.9; 1.5 ± 0.7; 1.5 ± 0.6 x10^9/l respectively (P=0.368).

In the viral group, 36.4% was classified as immunodepressed, whereas in alcoholic group was 45.5% and in other causes was 30.0%.

The behavior of the humoral immunity indicators according to the Child Pugh stage was different. With the worsening of the disease, the IgA increased, while the components of C3 and C4 complement decreased. The analysis of delayed intradermal hypersensitivity test for intestinal and respiratory antigens showed that patients in a Child Pugh B stage showed higher hipoergy than patients in stage C. The response for candidin and tetanus toxoid was as expected with a tendency to hipoergy and anergy as the disease got worse. The differences were significant for intestinal bacteria and tetanus toxoid as shown in Table 3.

The average values for the total lymphocyte count showed a decreasing tendency; 1.9 ±0.9 x 10^9/l in Child A, 1.5±0.4 x 10^9/l in B and it reached values below normal in Child C stage, 1.0±1.1 x 10^9/l.

Graph 1.
values of C4 in which a clear decrease of malnourished patients occurred with 0.16±0.08 g/L compared to the non-malnourished 0.21±0.09 g/L (P=0.025). The analysis of the results of the intradermic tests showed in the malnourished a higher immune compromise for candida and bacteria of the respiratory tract whose average nodule size was below 4mm. However in the non-malnourished, hipoergy was only seen in candida. Table 5.

The total lymphocyte count behaved in a similar way in both groups with 1.7±0.9x10^9 /L in the malnourished and 1.7±0.7x10^9 /L in the non-malnourished (P=0.368).

In relation to the state of immunocompetence, a higher percentage of immunodepressed in the undernourished was reflected, in comparison to the non-malnourished with 41.7% versus 28.6%, however this was not statistically significant (P=0.254).

**Discussion**

The main cause of the disease under study was viral, which corresponds to what has been reported for the geographic area to which our country belongs [19-23]. The sample was chosen randomly, only with certain inclusion criteria, that's why the majority was expected to be of this etiology. Studies published in Cuba in relation to the etiology of the disease show viral hepatitis as the most frequent, specifically HCV [24]. HBV plays an important role in other latitudes, however in the country, the application of vaccines against this virus in the population below 25 years and groups with greater risk have obtained a decrease in the incidence and prevalence in the last 15 years [25].

Less than 40% of patients were immunodepressed, this quantity can be considered as expected for a sample in which the majority maintained an acceptable liver function. It was interesting to see a major defect in the immune response to certain antigens like candida and bacteria of the respiratory tract.

Four characteristic types of infections have been described in cirrhotic patients, spontaneous bacterial peritonitis responsible for approximately 44%, urinary tract infection with 25%, pneumonia 15% and bacteria without a source in 5%. According to this order, it would be logical to think that a patient is more prone to infection of enteric origin than of respiratory [26]. The intestinal flora alterations in cirrhosis caused by the abnormal colonization of the small intestine increased the probability that gram negative aerobic bacteria invade the blood stream and cause infections of enteric origin [27,28]. However, the immune alterations observed in the study showed that the immunodepression for germs of the respiratory tract was more, which constitutes an interesting finding.

Patients with cirrhosis are exposed to respiratory tract infections due to various causes like the decrease in the cough mechanism, the risk of aspiration in patients with encephalopathy and the invasive procedures that facilitate nosocomial infections which along with the leukopenia secondary to the base disease, alcohol consumption and the exposure to specific community infections like tuberculosis or influenza increase the risk of infections which can end up being serious [7]. Streptococcus pneumoniae is the most frequent etiologic agent of pneumonias in cirrhosis to which we can add gram negative bacilli like Klebsiella pneumoniae and Haemophilus pneumoniae respectively [29].

| Sex       | n  | Age (years) | MAC (cm) | TST (mm) | SST (mm) | AMA (cm²) | AFA (cm²) |
|-----------|----|-------------|----------|----------|----------|-----------|-----------|
| **Child A** | 22 | 49.4±9.7    | 30.5±3   | 12.2±3.7 | 14.2±6.2 | 57.3±11.5 | 16.7±6.4 |
| **Child B** | 11 | 46.7±8.6    | 30.9±3.9 | 12.7±5.1 | 16.3±4.5 | 56.2±13.3 | 17.8±9.3 |
| **Child C ANOVA** | 5  | 43.8±6.6    | 26.7±3.6 | 10.6±4.2 | 10.4±2.7 | 43.7±8.5  | 13.0±6.4  |

**Table 4**: Anthropometric characteristics of patients according to sex and stage of the disease.

| Measurement immunoglobulins and complement (g/l) | Malnourished (48) | Non malnourished (28) | P Value |
|-------------------------------------------------|-------------------|-----------------------|---------|
| IgM                                             | 1.8±0.08          | 1.6±0.06              | 0.084   |
| IgG                                             | 7.9±0.07          | 7.9±0.05              | 0.702   |
| IgA                                             | 2.8±0.27          | 2.3±0.17              | 0.429   |
| IgE**                                            | 112.5±11.57       | 101.6±14.15           | 0.706   |
| C3                                              | 0.9±0.05          | 1.0±0.06              | 0.518   |
| C4                                              | 0.1±0.01          | 0.2±0.01              | 0.025*  |

**Intradermic (nodule size, mm)**

| Intestinal bacteria                              | 4.2±2.7           | 5.0±2.8               | 0.142   |
| Candidin                                         | 3.1±3.0           | 3.2±2.6               | 0.845   |
| Tetanic toxoid                                   | 4.9±2.8           | 5.8±2.6               | 0.191   |
| Respiratory bacteria                             | 3.4±2.7           | 4.1±3.0               | 0.352   |

C3 and C4: Complement components', Ig: immunoglobulin (A, M, G and E), Mean ± standard deviation, P: significance value *P<0.05, Mann-Whitney Test **IgE (kU/L)

**Table 5**: Indicators of humoral and cellular immunity in cirrhotic patients according to their nutritional state.
The pool of germs used as allergens in the study in their majority inhabit the nasopharinx of healthy individuals, however in immunocompromised patients with a disruption of the natural defense barriers, they become pathogens [30]. More studies would be needed to clarify if the immunodepression detected for these microorganisms is translated as a major incidence of this type of infections.

Candida albicans is an opportunistic germ with a high incidence in immunocompromised patients, the liver is one of the main responsible in the immunologic equilibrium, that is why any affectation in its function will compromise the immune state, therefore patients with cirrhosis are prone to these type of infections [31].

The levels of C4 of the complement showed different behaviors according to the causes of cirrhosis, their mean values in alcoholics and viral were lower which corresponds to that reported in other studies [1,22,33]. The inefficient liver synthesis of this protein has been associated with an alteration in the opsonization and bactericiding functions which is more evident in advanced stages of the disease. In the study, with the exception of one case, all patients in Child B and C stage were associated with these two causes (data not shown).

The cellular immunity was not different in the different causes; however, other studies have detected a higher energy in cirrhotics of alcoholic origin [34-37]. Alcohol consumption can cause lymphopenia and a decrease in the capacity of blastic transformation and cytotoxicity of the natural killer cells [38].

The reduction of C3 and C4 and the hemolytic activity of the complement by alternate way can be as a result of an increased consumption or a decreased production due to liver insufficiency, the magnitude of the damages has been negatively correlated with the levels of these complement factors [32].

It has been demonstrated that the decreased concentrations of C3 are due to the circulation of endotoxins in blood which has been significantly associated, independently to other variables like the major frequency of infections and major mortality. The acquired C3 deficiency in patients with liver cirrhosis could explain an increased risk of infections through a deficit in the capacity of opsonization, although it has been reported that this parameter doesn’t appear altered in a stable way in all patients [39-40]. In the study, results support this theory therefore with the worsening of liver function, a directly proportional decrease of these complement components were seen.

Of all immunoglobulins, only IgA showed substantial changes in its values, proportional to the progress of liver damage. IgA is the predominant antibody in seromucous secretions in the organism like saliva, tears, breast milk and respiratory tract secretions, gastrointestinal and genitourinary [41]. The increased levels of these immunoglobulins have been demonstrated in alcoholics and in patients with a worsening liver function. Evidence shows bacterial translocation as the main responsible, able to facilitate the passage of pathogen germs to the peripheral blood, the latter once there become associated with a series of molecular patterns like lipopolysaccharides, peptidoglycans and others, which are able to generate secretions from proinflammatory cytokines and the consequent activation of B cells and the synthesis of immunoglobulins [42].

The increased levels of IgA, IgG and the decrease in C3 has been seen in cirrhotics in the pre transplant period and has been associated to a higher risk of infection after surgery [43].

The majority of these patients show a decrease in cellular immunity, the activation and proliferation of incubated T lymphocytes with different immunostimulant products like the response to intradermic injection of antigens (late hypersensitivity) is more noteworthy in the non compensated [44-46]. The decrease in the total lymphocyte count and the depression in response to the intradermic tests seen in the study and its relation with the severity of the liver damage corresponds with other authors findings [47-50].

In a general way, the immunodepression was more in advanced stages of the disease which is attributed to a lower synthesis of factors related with innate immunity and to the hyper catabolism to which the organism has been submitted to by the disease.

Although the exact mechanism of immunologic incompetence in cirrhosis is not clear, various authors have described a close relationship between the alteration of cellular immunity and the malnutrition of cirrhotic patients. Malnourished cirrhotics are more prone to have infectious complications due to immunologic deficiencies in the humoral and cellular response [2-7].

Studies show that in the case of alcoholics, a close relationship exists between malnutrition and immunodepression, however in the viral etiology; the immunologic alterations are attributed more to the severity of the hepatocellular lesion and to the alterations that the viral infection prints over the immune system [47]. In the alcoholic liver disease, the likelihood for infections increases and this is due partly to a deficit of micronutrients, trace elements and vitamins with anti-oxidizing functions that increases the susceptibility of cellular membranes to the lipid peroxidation [51].

Other authors have observed other abnormalities of the immune system such as an increase in the activation of T cells and a decrease in B cells in patients with alcoholic liver cirrhosis, but these changes have been attributed to a direct effect of alcohol or of its metabolites [52,53].

It was expected that males were grouped in larger proportions than females in Child Pugh B or C and that the decrease of anthropometric indicators in these advanced stages of the disease were more evident in both sexes. This behavior is related to the catabolic effects that the progression adds and its complications [21].

Although all hypotheses evoke malnutrition as a possible responsible factor or contributing of immunologic disorders in this type of patients, the results obtained in the study were contradictory, better nutritional state and worse immunologic state have been reported in cirrhosis of viral cause and vice versa for alcoholics [47].

It wasn’t confirmed that a major immune involvement existed in malnourished patients, even though an important part of them were immunodepressed. Only a partial defect was detected in the humoral response due to the decrease in the C4 components of the complement. Interventional studies have demonstrated the betterment in the defensive mechanisms in this type of patients with nutritional support [54].

Of all elements considered in the investigation, it was the stage of progression of the disease that best evidenced the immune response. Child Pugh stage is considered the main and most consistent predictor of mortality in cirrhotic patients. According to D’Amico [55], patients in Child Pugh A stage have a low risk of dying therefore it would be useful in this group to find more predictors of decompensation more than that of mortality, of which the immunologic could be found.

Conclusions

In liver cirrhosis, the major contribution to the distorted immune response is the Child Pugh stage. Malnutrition maybe has a possible influence.
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