A Comparative Evaluation of Serum and Salivary Total Proteins and Immunoglobulins in Patients with Hepatitis A and Healthy Subjects

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Assessment of changes in total proteins level, serum and saliva IgG and IgA levels, serum IgM level, serum and saliva IgA/IgG ratio. The study was conducted on a group of 40 subjects, divided into 2 lots: the first lot consisting of 20 healthy individuals and the second consisting of 20 patients with hepatitis with hepatitis A virus (HAV). The levels of total proteins, serum and saliva IgG and IgA, serum IgM and serum and saliva IgA/IgG ratio have higher values in patients with hepatitis A, in comparison to healthy subjects, without necessarily exceeding the maximum admitted value. The results are significant from a statistical point of view. Due to the sensitivity and specificity of salivary anti-HAV IgM and IgG in patients with acute hepatitis A, compared with healthy subjects, there is a possibility of using salivary immunological tests instead of serum tests for the diagnosis and epidemiological study of HAV infection.

Keywords: hepatitis A, immunoglobulins, total proteins, saliva, serum

Hepatitis A is an acute liver disease caused by the infection with hepatitis A virus (HAV). The symptomatology is usually mild or even absent, especially in young people, and unlike hepatitis B or C hepatitis A does not cause chronic liver disease and is rarely fatal. When present, symptoms persist for approximately 8 weeks: most frequently nausea, vomiting, diarrhea, abdominal pain, fever syndrome and jaundice. The fulminant form of hepatitis A occurs rarely, more frequently after age 40 (1.1%), and less frequently in children (0.1%), resulting in acute hepatic cell necrosis and hepatic failure [1-4].

Hepatitis A is a worldwide spread disease. Its incidence is 1.4 million new cases per year. Hepatitis A is more frequent in developing countries (150 new cases/100,000 individuals/year), compared to industrialized countries. This difference may be attributed to the digestive transmission of HAV, which is influenced by socio-economic status and hygiene level [1,5].

Extrahepatic complications occur less frequently than in HBV or HCV infection and consist of cutaneous vasculitis that frequently involves the limbs. Cutaneous vasculitis is associated with cryoglobulinemia. HAV infection can cause immunological disorders in people who are predisposed to them. Therefore, chronic autoimmune hepatitis may have hepatitis A as a triggering factor. Other immunemediated manifestations include transverse myelitis, polynuropathy, optic neuritis, and arthritis [1].

Acute infection with HAV may cause non-specific inflammatory changes in the oral mucosa. The following aspects may be observed in a HAV patient: hyperemic aspect of the oral mucosa secondary to capillary dilatation and local trophic disorders, glossitis with atrophy of the lingual papillae, or exfoliative cheilitis on the border of the lips. In this context, the patient may report mouth drying and burning feeling involving the oral mucosa [6,7].

Experimental part

This study aimed to evaluate the changes in serum and salivary total proteins and immunoglobulins in patients with acute hepatitis A (AHA) compared to healthy individuals and the possibility of using salivary immunological tests for the diagnosis and epidemiological study of HAV infection instead of serum tests.

The study was conducted on a group of 40 subjects from the Infectious Diseases Department of MoND-Central Military Hospital, divided into two groups: group 1 consisting of 20 healthy individuals and group 2 consisting of 20 patients suffering from hepatitis with HAV. We formed 2 groups in which we determined the concentrations of some of the plasma protein and salivary components.

The individuals from group 1 were aged between 13 and 55 years (average age 33 years, σ=12.18). The patients from group 2 were aged between 15 and 50 years (average age 24.75 years, σ=9.88).

In order to establish the diagnosis, the presence of anti-HAV IgM in serum was tested. The inclusion criteria for group 2 was the presence of anti-HAV IgM, the mentioned group consisting, therefore, of patients with AHA. The healthy subjects had a negative anti-HAV IgM test result and were included in group 1. The patients with AHA had no severe clinical manifestations. All the cases had self-limiting evolution.

The blood parameters we determined were: (a) total proteins (g/dL), (b) IgG, IgA, IgM plasma immunoglobulins (g/L), (c) total anti-HAV IgG+IgM antibodies and anti-HAV IgM antibodies.

The following salivary parameters were determined: (a) total proteins (g/dL), (b) IgG, IgA, IgM immunoglobulins (g/L), (c) total anti-HAV IgG+IgM antibodies and anti-HAV IgM antibodies.

Determination of plasma immunoglobulins was performed using the immunonephelometric method, with BN 100 System, on the Dade Behring BN II analyzer, which automatically performs all the steps. During the immunochemical reaction with specific antibodies, serum proteins form immunocomplexes that disperse the light beam passing through the sample. The intensity of the
scattered light is proportional to the concentration of the protein to be examined in the sample. The result is appreciated in comparison to a standard of known concentration. The reference ranges for healthy adults are: IgG 7-16 g/L, IgA 0.7-4 g/L, IgM 0.4-2.3 g/L.

Determination of total proteins and protein fractions in plasma was performed by gel immunodiffusion method.

Determination of salivary immunoglobulins was achieved by simple radial immunodiffusion method.

Determination of salivary total proteins was performed using Lowry’s method: to 0.5 mL saliva, 1.5 mL distilled water and 1.5 mL 4% Na₂CO₃, were added. After stirring, 0.5 mL Folin-Ciocalteu reagent (CuSO₄, KI, Na tartrate in 2N Na₂CO₃ buffer), dilution 1:3, were added. The mixture was shaken, allowed to stand in the dark for 30 minutes, developing a blue color. Spekol RA-50 was read at λ = 620 nm. Optical density was converted to protein concentration (g/L) after the standard curve.

Results and discussions

Serum parameters

Because most plasma protein fractions – albumin, globulins – apart from plasma-derived immunoglobulins, are synthesized by hepatocytes, it is expected that in hepatic impairment changes in all of these components will occur. Liver synthetised proteins are often produced in low amounts in case of hepatopathy, but immunoglobulins vary, being influenced by inflammation and infection. Not only the liver function is involved in maintaining normal protein values, but also non-hepatic factors such as malnutrition, neoplasms or endocrine disease can influence the metabolism of plasma proteins.

Table 1 presents the average values and standard deviations of serum protein levels (g/dL), in healthy subjects compared to AHA patients.

Applying the t-test in order to determine whether there are significant differences between the two groups, we obtained p=0.01. If the p-value is below 0.05 the results are designated as statistically significant. Values below 0.01 indicate that the results are highly statistically significant.

The protein level increased significantly (p < 0.01) in patients with AHA, in comparison to healthy subjects, however, it did not exceed the highest accepted value (8.2 g/dL): 7.56 ± 0.49 g/dL in AHA patients, compared to 7.16 ± 0.51 g/dL in healthy subjects (Chart 1).

In table 2, there are represented comparatively the values of IgG, IgA, IgM serum concentrations, as well as of serum IgA/IgG ratio in healthy subjects, respectively in patients with AHA. The values are expressed in g/L.

The p-value, that indicates the statistical signification of the differences regarding the determined parameters, calculated by means of t-test is presented in table 3.
In chart 2 there are represented comparatively the values of IgG, IgA, IgM serum levels in healthy subjects and AHA patients.

IgG serum levels in healthy subjects had a 14.25 ± 1.91 g/L average value, while in AHA patients the average value was 16.47 ± 3.40 g/L. The differences were statistically significant, p < 0.01. The average value in AHA group was higher than the highest accepted value for IgG serum concentration, which is 16 g/L.

Serum IgA levels for the AHA patients were higher than those of the healthy subjects, the differences being statistically significant (p < 0.01): 3.45 ± 2.02 g/L, respectively 2.02 ± 0.95 g/L. Neither of the groups pointed out values out of the reference range: 0.7 – 4.0 g/L.

IgA/IgG ratio was significantly higher in the AHA group (0.21 ± 0.11) in comparison to the healthy subjects (0.15 ± 0.07), taking into consideration the fact that, even if both of its components increased, the increase of serum IgA was greater than the increase of serum IgG.

IgM also varied significantly: the average values were 1.41 ± 0.73 g/L for the healthy subjects and 3.82 ± 2.46 g/L for AHA patients, p < 0.01. The average value of IgM in AHA patients was higher than the highest accepted value for this parameter (2.3 g/L). The significant increase of serum IgM was to be expected, under the circumstances of the synthesis activation of anti-HAV IgM antibodies.

**Saliva parameters**

In table 4 there are represented comparatively the values of salivary total proteins, IgG and IgA immunoglobulines, as well as the ones of IgA/IgG ratio determined for the two groups that we studied.

Table 5 presents the values we determined in order to identify the significance of the differences between the two groups, applying the t-test.

In the groups we conducted the study on, we determined the following average values for salivary total proteins: 1.51 ± 0.59 g/L in healthy subjects and 2.29 ± 1.96 g/L in AHA patients. The difference between the groups is statistically significant (p < 0.01).

Salivary IgG concentration for the AHA patients was also significantly increased compared to healthy subjects: 3.01 ± 0.78 mg/dL, compared to 2.41 ± 0.42 mg/dL, p < 0.01.

For group 1 – healthy subjects, the average value of salivary IgA was 10.38 ± 4.51 mg/dL. In the AHA group, the average value was 39.01 ± 22.51 mg/dL, significantly higher, p < 0.01.

Salivary IgA/IgG ratio was mainly influenced by IgA concentration; the ratio increased significantly (p < 0.01) in the AHA group, compared to the healthy group, because IgA had a greater increase than IgG. The average values were: 4.51 ± 2.16 in healthy subjects and 13.71 ± 8.79 in AHA patients.

Therefore, for anti-HAV IgM there was a 90% specificity and a 100% specificity. For total anti-HAV immunoglobulins (IgM+IgG) there was a 95% sensitivity and a 90% specificity.

Searching the published literature, we found many studies that analyze the possibility of using oral fluid as a...
possible replacement for immunological serum tests in HAV infection [8].

Regarding the presence of viral RNA-HAV, the data in the literature are contradictory and there is noted the need for longitudinal studies to accurately analyze its presence in saliva and to determine the host-dependent factors and viral factors that influence the salivary viral presence in hepatitis A, B or C [9,10].

Our results are consistent with other similar studies. Ahmed M et al report a 89.06% specificity of anti-HAV Ig salivary tests for AHA. They also state, with regard to salivary tests, that a positive result implies a 96.34% chance that the individual will suffer or had suffered a HAV infection and a 3.66% chance of a false-positive result. A negative result implies a 72.75% chance that the individual had not suffered or is not currently suffering from a HAV infection, and a 27.25% chance of a false negative result [11].

Amando Leon et al state that despite the large number of studies investigating orally the AHA diagnosis, the frequency and pattern of salivary markers in oral fluid remains unknown. They conducted a longitudinal study to analyze the dynamics of HAV viral markers in saliva, as compared to serum. They concluded that maximum diagnostic accuracy using salivary anti-HAV Ig is achieved 30-90 days after the onset of AHA [12].

Reticence to serum tests in the general population is known. This is explained by the stress caused by the venous puncture and by the associated costs. Ahmed M et al suggest the use of salivary tests in favor of overcoming these inconveniences [11].

Minor differences in the literature regarding the accuracy of the anti-HAV Ig salivary testing for AHA diagnosis can be due to the lack of special saliva harvesting devices. Even under these conditions, salivary tests are viable to be used on a large scale. The possibility of using special saliva sampling devices should be analyzed, but this is unlikely, given the increased degree of difficulty and costs [13,14].

Conclusions

Serum total proteins level increases significantly in AHA patients, compared to healthy subjects, although it does not exceed the upper normal limit.

Serum IgG, IgA and IgM concentrations are significantly higher in AHA patients, compared to healthy subjects. IgG and IgM exceeding the highest accepted value. Because IgA increases more than IgG, the IgA/IgG serum ratio is significantly higher in AHA.

Salivary total proteins, IgA and IgG concentrations are significantly increased in AHA.

As in the serum, the salivary IgA/IgG ratio is mainly influenced by the IgA concentration; the ratio increases significantly in AHA compared to healthy subjects, because IgA has a greater increase than IgG.

The sensitivity and specificity of the tests used for detecting anti-HAV antibodies in saliva is high and the tests can be used both for diagnosis and for the epidemiological study of HAV infection in place of serum tests.

Currently there are few salivary tests for emergency or routine diagnosis, including tests for HIV and HCV infection. Given that anti-HAV Ig salivary tests are reported to have a high specificity and sensitivity for AHA, lower invasiveness compared to serum testing, as well as lower costs, consideration should be given to the suitability of using such tests to reduce the costs of immunization in developing countries with endemic problems in order to increase the effectiveness of HAV vaccination but also as a method of diagnosis and epidemiological surveillance.

References