# Salivary and Serum Biochemical Alterations in Patients with Acute Viral Hepatitis

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*Viral hepatitis represents a major health problem worldwide. Approximately 1.4 million people are infected with hepatitis A virus every year, although given that most of the cases evolve asymptomatically the real number could be even higher. At the same time, hepatitis B virus affects up to 30% of the world population and represents one of the main causes of cirrhosis and hepatocellular carcinoma. Thus, it is very important to understand the physiopathology of viral hepatitis A and B not only for the diagnosis, but also for the therapeutic protocol. The present research aimed to determine if HAV and HBV can alter serum and salivary levels of total protein and of 2 important electrolytes: calcium and potassium.*

*Keywords: hepatitis A virus, hepatitis B virus, saliva*

Hepatitis A is an infectious disease that in most cases is asymptomatic in adults (in up to 95% of the cases). When present, symptoms include nausea, fever, fatigability, vomit, diarrhea or appetite decrease. However, in severe situations, hepatitis A can lead to hepatic failure. The hepatitis A virus (HAV), a member of the *Picornaviridae* family is found in feces, therefore the main route of transmission is the fecal-oral path. Other ways of contracting the virus include the consumption of infected water or food, as well as direct contact with a contagious person. Incubation period varies between 2 and 6 weeks and recovery can last up to 6 months. During the acute stage of the viral infection, anti-HAV IgM antibodies can be identified (following this stage, the main type of antibodies is represented by IgG) [1].

On the other hand, hepatitis B represents a more serious threat. Up to 40% of the men and 15% of the women infected with hepatitis B virus (HBV) can develop liver cirrhosis or hepatocellular carcinoma that lead to death. Romania has the second highest incidence for hepatocellular carcinoma in Europe [2]. It is estimated that almost one third of the world population is or has been infected with HBV, although the very efficient vaccine anti-HBV has reduced the prevalence of this disease [3, 4]. Apart from the symptoms described in HAV infection, 1% of HBV patients can display extrahepatic manifestations such as vasculitis, aplastic anemia or glomerulonephritis [5].

Meanwhile, saliva has proven to be an exceptional diagnosis and monitoring fluid. Non-invasive, this complex mixture of organic and inorganic components offers advantages such as the requirement of a low quantity for analysis, correlation with blood concentrations, as well as easy collection [6]. Thus, more and more studies focus on the use of this exceptional fluid as a serum substitute.

In this context, our study aimed to measure both serum and salivary levels of some parameters, such as total protein, calcium and potassium in patients with acute hepatitis A and B, taking into consideration that most of the studies in the field have focused on chronic liver afflictions.

## Experimental part

### *Patient selection*

The present research included a total of 51 patients. The HA group comprised of 18 patients (15 men and 3 women) diagnosed with acute viral hepatitis A, while the HB group consisted of 16 patients (14 men and 2 women) diagnosed with acute viral hepatitis B. Seventeen healthy subject, with no prior history of hepatitis were included in our study as a control group.

Patients were diagnosed with acute viral hepatitis A and B after the determination of viral hepatitis serum markers such as anti-HAV IgM, anti-HBc IgM, AgHBe, anti-HBe, anti-HBc, Ag-HVD, anti-HDV, anti-HCV, RNA-HCV. Patients with HAV were anti-HAV IgM positive, AgHBs negative, anti-HBc IgM negative, AgHBe negative, anti-HCV and RNA-HCV negative, while patients with HBV were anti-HVA IgM negative, anti-HCV and RNA-HCV negative and AgHBs and anti-HBc IgM positive. Clinical parameters such as age, sex, geographical area, weight and height were noted for all the subjects included.

### *Sample collection*

Salivary and serum samples were collected in the morning, between 8.30 and 9.30. Patients were asked not to eat for 12 h prior to the collection and to avoid drug administration 24 h preceding the sampling, excepting patients with mandatory medication. 30 min before the collection, patients were required to rinse their mouths with distilled water. Smoking as well as physical effort were forbidden before the sampling.

15 min after blood was collected by venous puncture, unstimulated whole saliva was collected in graduated sterile tubes. The first 3 mL were thrown away. If the volume was smaller than 3 ml, sample collection was repeated 2 or even 3 times, after 10 minutes breaks. All the subjects were relaxed and requested not to swallow during collection. Immediately after the samples were obtained, the tubes were placed in ice recipients. Saliva samples were centrifuged for 20 min at 6000 rotations/min.

Total protein (TP), total and ionic calcium (Ca) and potassium (K) were determined in serum and TP, Ca and K

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in saliva. For serum, we also determined the albumin percentage from total proteins, knowing that almost half of the calcium is bound to these particular proteins. Ionic concentrations were determined using dry chemistry analyzers (VITROS 250 for serum biochemistry and VITROS 750XRC for salivary biochemistry). Serum total proteins concentrations were determined using a gel immuno-diffusion method. Meanwhile, salivary concentrations for total protein were determined using the Lowry method: 1.5 mL of distilled water and 1.5 mL $Na_2CO3$ 4% were added to 0.5 mL of saliva. The mixture was stirred and 0.5 mL of Folin-Ciocalteu reactive were added. After stirring, the new mixture was left for 30 min in the dark. Optic density was read using Spekol RA-50 to a $\lambda$=620nm and transformed in protein concentration (g/L) using an etalon-curve. Salivary *p*H and flux were also determined.

*Statistical analysis*

Results were expressed as mean± standard deviation (SD) for each lot. Statistical analysis was performed using ANOVA and Student t-test. A p-value < 0.05 was considered statistically significant, while p-values < 0.01 were considered to have high statistical significance.

## Results and discussions

*Clinical data*

Mean age (± standard deviation) at inclusion for the control group was of 32.41 ± 13.74 years. Meanwhile, mean age at inclusion was of 24.05 ± 9.08 years for the HA group and of 23.87 ± 7.59 years for HB group (tables 1, 2, and 3).

*Serum parameters*

Our results showed significantly increased levels of serum ionic calcium in both VHA patients (p=0.001) and VHB (p=0.003) *versus* the control group. However, no such significance was found regarding total calcium when comparing to the control group. Nonetheless, a statistically significant increase of total calcium was found in patients with VHB comparing to patients with VHA (p=0.04). Also, a positive correlation between ionic calcium and total calcium was found in the HB group (p=0.01). The individual concentrations, as well as the means ± standard deviations can be found in tables 4,5 and 6.

Potassium levels were significantly lower in VHA patients *versus* the controls (p=0.04). Nevertheless, no significant differences were found for the VHB patients. Potassium, the main cation in the cell was found to have decreased concentrations in patients with dietary

| | Sex | Geographical area | Age (years) | Weight (kg) | Height (cm) |
|---|---|---|---|---|---|
| 1. | M | urban | 25 | 80 | 190 |
| 2. | M | rural | 13 | 43 | 155 |
| 3. | M | urban | 19 | 60 | 170 |
| 4. | M | urban | 20 | 60 | 170 |
| 5. | M | urban | 20 | 69 | 170 |
| 6. | M | urban | 42 | 80 | 170 |
| 7. | M | rural | 20 | 80 | 180 |
| 8. | F | urban | 51 | 65 | 160 |
| 9. | M | urban | 61 | 80 | 177 |
| 10. | M | urban | 31 | 65 | 169 |
| 11. | F | urban | 35 | 76 | 172 |
| 12. | F | urban | 36 | 71 | 176 |
| 13. | F | urban | 31 | 73 | 177 |
| 14. | M | rural | 20 | 71 | 180 |
| 15. | F | urban | 32 | 80 | 175 |
| 16. | F | urban | 53 | 68 | 173 |
| 17. | M | urban | 42 | 72 | 178 |
| **Mean** | | | **32.41** | **70.17** | **173.05** |
| **Standard deviation** | | | **13.74** | **9.79** | **7.91** |

**Table 1**
CLINICAL DATA FOR THE CONTROL GROUP

| | Sex | Geographical area | Age (years) | Weight (kg) | Height (cm) |
|---|---|---|---|---|---|
| 1. | F | urban | 26 | 54 | 160 |
| 2. | M | urban | 27 | 75 | 180 |
| 3. | M | rural | 19 | 58 | 170 |
| 4. | M | urban | 22 | 55 | 175 |
| 5. | M | urban | 21 | 78 | 175 |
| 6. | M | rural | 32 | 75 | 170 |
| 7. | M | urban | 21 | 70 | 170 |
| 8. | M | urban | 44 | 72 | 170 |
| 9. | M | urban | 20 | 65 | 170 |
| 10. | M | urban | 19 | 60 | 168 |
| 11. | M | rural | 20 | 70 | 173 |
| 12. | M | urban | 15 | 63 | 170 |
| 13. | M | rural | 16 | 68 | 172 |
| 14. | F | urban | 21 | 71 | 175 |
| 15. | F | urban | 50 | 65 | 168 |
| 16. | M | rural | 17 | 68 | 173 |
| 17. | M | rural | 19 | 70 | 178 |
| 18. | M | urban | 24 | 72 | 175 |
| **Mean** | | | **24.05** | **67.16** | **171.77** |
| **Standard deviation** | | | **9.08** | **6.71** | **4.32** |

**Table 2**
CLINICAL DATA FOR THE HA GROUP

| | Sex | Geographical area | Age (years) | Weight (kg) | Height (cm) |
|---|---|---|---|---|---|
| 1. | M | rural | 23 | 70 | 175 |
| 2. | M | urban | 28 | 70 | 175 |
| 3. | M | urban | 21 | 70 | 185 |
| 4. | F | urban | 26 | 60 | 170 |
| 5. | M | urban | 50 | 65 | 167 |
| 6. | M | rural | 23 | 63 | 170 |
| 7. | M | urban | 20 | 75 | 180 |
| 8. | M | rural | 21 | 60 | 169 |
| 9. | F | urban | 28 | 66 | 172 |
| 10. | M | rural | 21 | 63 | 173 |
| 11. | M | urban | 19 | 72 | 180 |
| 12. | M | rural | 20 | 75 | 179 |
| 13. | M | urban | 20 | 65 | 170 |
| 14. | M | urban | 20 | 62 | 171 |
| 15. | M | urban | 24 | 60 | 165 |
| 16. | M | rural | 18 | 68 | 170 |
| **Mean** | | | **23.87** | **66.5** | **173.18** |
| **Standard deviation** | | | **7.59** | **5.09** | **5.43** |

**Table 3**
CLINICAL DATA FOR THE HB GROUP

**Table 4**
SERUM CONCENTRATIONS OF THE BIOCHEMICAL PARAMETERS IN THE CONTROL GROUP

| | Total Ca (mg/dl) | Ionic Ca (mg/dl) | K (mmol/l) | Total protein (g/dl) | Albumins (%) |
|---|---|---|---|---|---|
| **1** | 10.2 | 4.7 | 4.1 | 7.2 | 64.6 |
| **2** | 9.4 | 3.9 | 4.2 | 7.7 | 65.1 |
| **3** | 9.7 | 4 | 4.7 | 6.6 | 52.3 |
| **4** | 9.2 | 3.7 | 4.5 | 7.9 | 57.2 |
| **5** | 9 | 3.5 | 4.8 | 7.4 | 56.8 |
| **6** | 8.8 | 3.9 | 4.8 | 8.1 | 55.4 |
| **7** | 9.9 | 3.7 | 4.4 | 7.3 | 58.1 |
| **8** | 8.9 | 3.9 | 4.9 | 6.5 | 60.5 |
| **9** | 9.4 | 3.6 | 4.6 | 6.3 | 64.1 |
| **10** | 9.2 | 3.5 | 5 | 7 | 59.5 |
| **11** | 8.8 | 3.6 | 4.8 | 7.5 | 65.6 |
| **12** | 10.1 | 4.3 | 4.5 | 7.1 | 64.2 |
| **13** | 9.7 | 4.2 | 4.8 | 6.5 | 66 |
| **14** | 9.6 | 3.6 | 4.2 | 7.3 | 65.3 |
| **15** | 10 | 4.4 | 4 | 6.6 | 59.6 |
| **16** | 9.6 | 3.9 | 4.8 | 6.7 | 62.6 |
| **17** | 9.8 | 4.2 | 4.9 | 8 | 62.1 |
| **Mean** | **9.49** | **3.92** | **4.59** | **7.16** | **61.12** |
| **SD** | **0.45** | **0.35** | **0.31** | **0.57** | **4.11** |

**Table 5**
SERUM CONCENTRATIONS OF THE BIOCHEMICAL PARAMETERS IN THE HA GROUP

| | Total Ca (mg/dl) | Ionic Ca (mg/dl) | K (mmol/l) | Total protein (g/dl) | Albumins (%) |
|---|---|---|---|---|---|
| **1** | 9.4 | 3.5 | 4.5 | 7.6 | 58.7 |
| **2** | 9.4 | 4.1 | 3.9 | 6.3 | 60.8 |
| **3** | 9.5 | 4.3 | 4.6 | 7.7 | 58.8 |
| **4** | 10.2 | 4.6 | 4.8 | 7.9 | 55.2 |
| **5** | 9.6 | 4.3 | 4.6 | 8.5 | 42.4 |
| **6** | 9.5 | 4.2 | 4.2 | 6.9 | 66.7 |
| **7** | 9.6 | 4.3 | 5 | 7.2 | 62.8 |
| **8** | 9.4 | 4.2 | 4.2 | 8.2 | 60.1 |
| **9** | 9.5 | 4 | 4.5 | 6.5 | 56.3 |
| **10** | 8.9 | 4 | 4.6 | 6 | 65.5 |
| **11** | 10.2 | 4.6 | 4.2 | 7.5 | 55.2 |
| **12** | 10 | 4.7 | 4.9 | 7 | 60.1 |
| **13** | 9.6 | 4.1 | 4.1 | 7.1 | 61.7 |
| **14** | 9.8 | 4.2 | 4.4 | 7.3 | 59.5 |
| **15** | 9.8 | 4.2 | 4.5 | 5.5 | 62.8 |
| **16** | 10.1 | 4.5 | 4.6 | 7.8 | 60.6 |
| **Mean** | **9.66** | **4.24** | **4.48** | **7.19** | **59.20** |
| **SD** | **0.35** | **0.29** | **0.30** | **0.81** | **5.52** |

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|---|---|---|---|---|---|
| **1** | 9.4 | 3.5 | 4.5 | 7.6 | 58.7 |
| **2** | 9.4 | 4.1 | 3.9 | 6.3 | 60.8 |
| **3** | 9.5 | 4.3 | 4.6 | 7.7 | 58.8 |
| **4** | 10.2 | 4.6 | 4.8 | 7.9 | 55.2 |
| **5** | 9.6 | 4.3 | 4.6 | 8.5 | 42.4 |
| **6** | 9.5 | 4.2 | 4.2 | 6.9 | 66.7 |
| **7** | 9.6 | 4.3 | 5 | 7.2 | 62.8 |
| **8** | 9.4 | 4.2 | 4.2 | 8.2 | 60.1 |
| **9** | 9.5 | 4 | 4.5 | 6.5 | 56.3 |
| **10** | 8.9 | 4 | 4.6 | 6 | 65.5 |
| **11** | 10.2 | 4.6 | 4.2 | 7.5 | 55.2 |
| **12** | 10 | 4.7 | 4.9 | 7 | 60.1 |
| **13** | 9.6 | 4.1 | 4.1 | 7.1 | 61.7 |
| **14** | 9.8 | 4.2 | 4.4 | 7.3 | 59.5 |
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| **Mean** | **9.66** | **4.24** | **4.48** | **7.19** | **59.20** |
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**Table 6**
SERUM CONCENTRATIONS OF THE BIOCHEMICAL PARAMETERS IN THE HB GROUP

deficiencies or in subjects with urinary or gastrointestinal loss [7]. Hypokalemia (low serum levels of K) can decrease muscle strength and in severe cases lead to paralysis or cardiac arrhythmia. Research has focused more on potassium levels in chronic hepatic diseases and results showed that the more severe is the liver affliction, the more important is the hypokalemia which in most cases is caused by medication [8].

Total protein were found to have significantly higher levels in HAV patients *vs* the control group (p=0.03), but no such significance has been detected regarding HBV patients. Meanwhile, although we could not find a statistical significance, our results show that albumin levels are decreased in hepatitis patients comparing to the control group (individual concentrations in tables 4, 5 and 6).

In this context, the higher level of ionic calcium in patients with acute viral hepatitis A and B compared to controls could be explained by the decreased concentration of albumins. A lower quantity of these proteins will bind less calcium, hence increasing ionic calcium without any effects of total calcium. Similar results regarding total serum calcium have been found by studies focusing on chronic hepatitis [9].

*Salivary parameters*

Salivary Ca levels were found to be significantly lower in both VHA and VHB patients *versus* controls [mean ±SD (1.75 ± 0.43 mg/dL *vs* 2.04 ± 0.46 mg/dL, p=0.03, respectively 1.74 ± 0.36 mg/dL *vs* 2.04 ± 0.46 mg/dL, p=0.02)]. Significantly decreased salivary levels of potassium were found in both groups of hepatitis patients when compared to the healthy subjects [mean ± SD (13.11 ±4.01 mmol/L for HA group *vs* 21.58 ± 8.41 mmol/L in controls, p=0.0003 and 14.08 ± 3.14 mmol/L in HB patients *vs* 21.58 ±8.41 mmol/L in controls, p=0.001)]. Also, increased levels of salivary proteins have been detected in both VHA and VHB patients *versus* the control group [mean ± SD (2.41 ±1.00 g/L *vs* 1.52 ± 0.6 g/L, p=0.002, respectively 2.56 ± 1.14 g/L *vs* 1.52 ± 0.6 g/L, p=0.001)] (fig. 1).

Although we can't speak of hyposalivation, salivary flux was found to be decreased in patients with VHA (0.40±0.18 mL/min; p=0.0001) and patients with VHB (0.46±0.18 mL/min; p= 0.002) compared to healthy subjects (0.60±0.22 mL/min) (fig. 2).

Taking into account that salivary *p*H varies proportionally with salivary flux, our results showed a *p*H decrease in both the hepatitis groups compared to the controls (mean

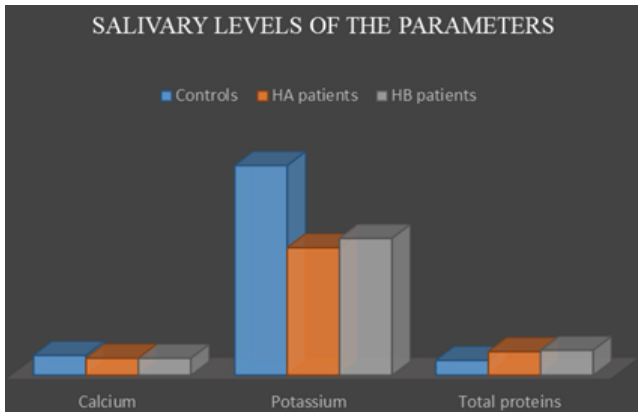


Fig. 1. Salivary levels of Ca, K and total proteins

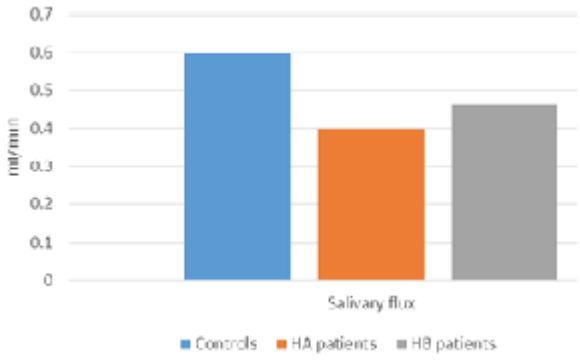


Fig. 2. Salivary flux

*p*H in controls was of 6.85±0.77; 6.25±0.45 for VHA, p= 0.004; 6.44±0.63 for VHB, p=0.04) (fig. 3).

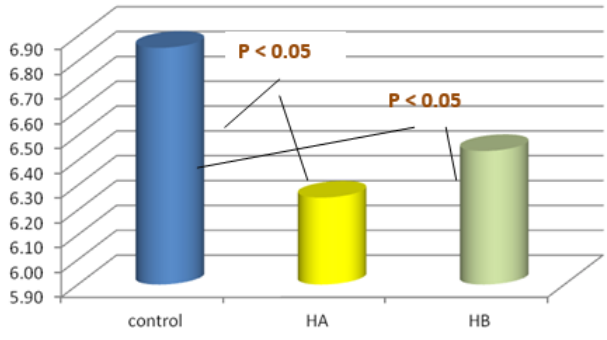


Fig. 3. Salivary *p*H

| | TP | Ca | K |
|---|---|---|---|
| **Control group** | | | |
| **Salivary flux** | 0.33 | -0.41 | -0.42 |
| **Salivary pH** | -0.03 | -0.17 | -0.27 |
| **HA group** | | | |
| **Salivary flux** | -0.18 | -0.16 | 0.35 |
| **Salivary pH** | -0.48 | 0.22 | 0.54 |
| **HB group** | | | |
| **Salivary flux** | -0.26 | 0.005 | 0.35 |
| **Salivary pH** | -0.12 | 0.13 | 0.54 |

**Table 7**
STATISTICAL CORRELATIONS BETWEEN SALIVARY LEVELS OF THE DETERMINED BIOCHEMICAL PARAMETERS AND SALIVARY FLUX AND *p*H

Analyzing the salivary levels determined for the three parameters and the results of our study regarding salivary flux and *p*H in all the subjects, our research team discovered a positive correlation between salivary flux and salivary levels of Ca (table 7). Previous studies show that salivary calcium and salivary flux variate proportionally, hence supporting the results of the present work [10]. The decreased salivary flux could explain the lower calcium levels in saliva in patients with acute viral hepatitis A and B, although the higher protein level could also provide an explanation.

We also found a negative correlation between total protein and *p*H in the control group. However, no such result could be obtained regarding TP and salivary flux, similar results being revealed by previous studies monitoring the relationship between these 2 important salivary parameters [11]. In addition, the only positive correlation found between salivary and serum levels in all groups was for potassium (p=0.04 in controls, p=0.01 in HA group and p=-0.03 in HB group).

## Conclusions

To sum up, patients with acute viral hepatitis A and B present significant electrolytic alterations both in serum and saliva. Further investigation of the physyopathological mechanisms involved in these disturbances is needed for an improved therapeutic protocol.

*Acknowledgements: All authors contributed equally to the present work. The authors declare no conflict of interest.*

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Manuscript received: 23.03.2017