Transmission of Hepatitis-B Virus Through Salivary Blood Group Antigens in Saliva
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ABSTRACT
Objective: To determine an association between transmission of hepatitis B virus and secretor and non-secretor status of salivary blood group antigens.
Study Design: Cross-sectional, analytical study.
Place and Duration of Study: The Department of Physiology and Division of Hepatology, College of Medicine, King Khalid University Hospital, King Saud University, Riyadh, Kingdom of Saudi Arabia, from 2007 to 2009.
Methodology: Eighty eight known patients, who were positive for Hepatitis B Surface Antigen [HBsAg] were recruited. Saliva was collected for investigating the secretor and non-secretor status by using blood typing kit number Kemtec Educational Science USA. Hepatitis B Surface antigen test was performed on Enzyme Linked Immunosorbent Assay technique. Polymerase chain reaction [PCR] on saliva was also carried out in High Performance Thermal Cycler-Palm-Cycler\textsuperscript{TM} [Corbett Life Science, Sydney, Australia] and enzymatic amplification of extracted viral DNA was performed using primers covering the promoter of the core region of HBV.
Results: Out of the 88 subjects, 61 belong to blood group O, 20 to A and 7 subjects to blood group B. Fifty subjects were secretors [salivary blood group antigens positive] and 38 subjects were non-secretors [salivary blood group antigens negative]. Among core gene positive 25 (69.4%) were secretors and 11 (30.6%) were non-secretors. However, in core gene negative 25 (48.1%) were secretors and 27 (51.9%) were non-secretors.
Conclusion: The result shows an association [p=0.047] between secretor and non-secretors status of the salivary blood group antigens with core gene positive and core gene negative.

Key words: Saliva. Salivary blood group. Secretor. Non-secretors. Hepatitis B.

INTRODUCTION
Hepatitis B virus infection is a leading health care problem which constitutes a global medical challenge. Introduction of recent methods for diagnosis, improved the health and hygienic standards, screening of blood donors together with the availability of vaccine has greatly helped in reducing the prevalence of viral hepatitis. But, still hepatitis B virus (HBV) infection is a worldwide public health care problem. It is estimated that globally 300-420 million people are chronically infected with hepatitis B (HB) affecting 5-7% of total world’s population.\textsuperscript{1,2} There are about 2 billion people [nearly a third of the world’s population] infected by HBV and among these more than 350 million have chronic infection. Patients with chronic infection have a high mortality risk for hepatic cirrhosis or liver cancer. Infection with hepatitis-B virus has been a significant cause of morbidity claiming more than a million lives every year.\textsuperscript{3}

The main routes of transmission are contact with infected blood or other body fluids. In developed countries the sexual contact is responsible for 30% of infections and is the main cause of HBV transmission.\textsuperscript{4,5} Health professionals, such as surgeons, pathologists, dialysis and chemotherapy technicians have a high risk of acquiring HBV infections through small skin lesions or accident with instruments that cut or perforate.\textsuperscript{6} Hepatitis B virus has also been found in various body fluids including serum, saliva, nasopharyngeal fluid, urine, tears, semen and breast milk.\textsuperscript{7-14} For the transmission of hepatitis B virus blood, body fluids and sexual route have been paid greater attention. However, to the best of our knowledge, globally no study has been conducted yet, to demonstrate the role of secretor and non-secretor status of salivary blood groups and transmission of hepatitis B virus infection.

Therefore, the aim of this study was to determine an association between transmission of hepatitis B virus and secretor and non-secretor status of salivary blood group antigens.
Salivary blood group and hepatitis B

METHODOLOGY

The present study was conducted in the Department of Physiology and Division of Gastroenterology, College of Medicine, King Khalid University Hospital, King Saud University, Riyadh, Kingdom of Saudi Arabia, from 2007 to 2009. College of Medicine Research Centre and Ethical Committee approved the study, and patients’ consent was obtained. In this study, 88 volunteer, known patients suffering from hepatitis B were recruited from the Division of Hepatology, King Khalid University Hospital and Division of Hepatology, Military Hospital Riyadh, Saudi Arabia. Subjects with any known malignancy, Acquired immune deficiency syndrome (AIDS) and drug addicts were excluded from the study.

After getting a clinical history, 5-10 ml of fresh saliva sample was collected in a disposable container with a specific patient identification code number. Saliva sample was kept at 4°C prior to centrifuge for 15 minutes at 1000 g. Supernatants was boiled at 100°C for 25 minutes and was stored at 20°C prior to testing. The time from collection of saliva sample to storage was kept keeping in mind that, it would not exceed 5 hours. Secretor and non-secretor status of the saliva was investigated by using blood typing kit number 11, Kemtec Educational Science, USA, developed to identify Lewis (Le) blood group antigen phenotypes Le (a -ve b +ve) secretor, and Le (a +ve b -ve) non-secretor blood type.

For hepatitis B surface antigen 15 ml of venous blood was collected from all the selected subjects by venepuncture and the sample of blood was immediately transferred to a plain plastic tube. Each tube was labelled with the subject identification code number and Hbs antigen test was performed on Enzyme Linked Immunosorbent Assay technique. DNA was extracted from 100 µl of saliva using Puregene DNA purification kit (Gentra Systems, Minneapolis, Minnesota, USA) following the manufacturer’s instruction and recommendations. DNA was re-suspended in 20 µl hydration solution and a master mix (10 mM Tris-HCL pH 8.3, 50 mM KCL, 1.5 mM MgCl₂, 0.01% gelatin), 0.2 mM of each of dNTPs and a master mix (10 mM Tris-HCL pH 8.3, 50 mM KCL, 1.5 mM MgCl₂, 0.01% gelatin), 0.2 mM of each of dNTPs (Amersham Bioscience, Piscatway NJ, USA), 20 picomoles of P1 (+) and Core 2 (-) primers and 1.25 units of AmpliTag® DNA polymerase (Applied Biosystems, Foster City CA, USA). The second round was performed with 20 picomoles each of P1 (+) and T 718 (-) primers using 2.5 µl from the first round PCR product as template. Before amplification was carried out, all the samples were incubated at 95°C for 5 min, followed by denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute and extension at 72°C for 1 minute for a total of 35 cycles for round 1, and 30 cycles for round two. A PCR reaction mixture was added to each run with no DNA template which serves as negative control. A great care was taken to avoid contamination throughout the procedure as extraction of nucleic acids, preparation of mixtures, thermal cycling and post PCR analysis were done on separate areas. No evidence of DNA carryover was observed under these conditions. Ten µl of the amplified products were electrophoresed on 2% agarose gel, stained with ethidium bromide (1µg/ml) and visualized under UV illumination. The detection of HBV and β-globin gene in saliva with molecular weight markers is shown in Figure 1 and 2 respectively.

SPSS-17 software program was used for data entry and analysis. Chi-square test was applied to test an association between secretor and non-secretor status of salivary blood groups with core gene positive and core gene negative. Results were presented as numbers and percentage and level of significance was achieved at p=0.05.

Table I: The sequence of primers used in the study.

<table>
<thead>
<tr>
<th>Primer</th>
<th>NT position</th>
<th>Sequence 5’ to 3’</th>
<th>Amplicon size</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 [+]</td>
<td>1822-11842</td>
<td>20 S TTT TCA CCT CTG CCT AAT CA 3' 661 bp</td>
<td>Genther et al.16</td>
</tr>
<tr>
<td>Core 2 [-]</td>
<td>2482-2462</td>
<td>20 S CCS ACC TTA TGA GTC CAA GG 3'</td>
<td>Suwannakan et al.16</td>
</tr>
<tr>
<td>P1 [-]</td>
<td>1822-11842</td>
<td>20 S TTT TCA CCT CTG CCT AAT CA 3' 661 bp</td>
<td></td>
</tr>
<tr>
<td>Core gene round 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T718 [-]</td>
<td>2286-2266</td>
<td>20 S GGA GTG CGA ATC CAC ACT CC 3' 465 bp</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Detection of hepatitis B virus (HBV) in saliva. This is a representative gel of the PCR products obtained using primers directed against the core region of HBV. Lane M, 4X Hae III DNA molecular weight marker; lane 1, a positive control using pAM6 plasmid that contains the whole genome of HBV, lanes 2 to 11, DNAs extracted from saliva of different patients; lane 12, a negative control. Arrow is the molecular weight of the PCR product.
As shown in Table II the total number of cases is 88. Out of these, 61 had blood group O, 20 had blood group A and 7 had blood group B. In blood group O, 23 were secretors and 38 were non-secretors. In blood group A, 20 were secretors and none of the case was non-secretor. Similarly, in blood group B, 7 were secretors and none of the case was non-secretor. Moreover, in blood group O, 24 were core gene positive and 37 were core gene negative and in blood group A, 8 were with core gene positive and 12 were core gene negative and in blood group B, 4 were core gene positive and 3 were core gene negative.

Table II: Distribution of core gene positive and negative, and secretor and non-secretor status among the participants.

<table>
<thead>
<tr>
<th>Blood group</th>
<th>No. of cases</th>
<th>Core gene positive</th>
<th>Core gene negative</th>
<th>Secretor</th>
<th>Non-secretor</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>61</td>
<td>24</td>
<td>37</td>
<td>23</td>
<td>38</td>
</tr>
<tr>
<td>A</td>
<td>20</td>
<td>8</td>
<td>12</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>36</td>
<td>52</td>
<td>50</td>
<td>38</td>
</tr>
</tbody>
</table>

Results

Hepatitis B is a major leading health care problem globally including the Arab world, where countries reach at a large proportion of their population with hepatitis B. Vaccination, which is reducing the infection rate, particularly in the Saudi Arabia. But still Saudi Arabia is considered as an area of endemic hepatitis B virus (HBV) infection. Despite the apparent homogeneity of the Saudi population, the prevalence rate of HBV varied among the population.

Table III: The association of core gene among the various blood groups with positive and negative salivary blood group antigens (secretor and non-secretor status).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Secretor</th>
<th>Non-Secretor</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Group</td>
<td>Core gene positive</td>
<td>Core gene negative</td>
<td>Core gene positive</td>
</tr>
<tr>
<td>O</td>
<td>13</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>A</td>
<td>8</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>25</td>
<td>11</td>
</tr>
</tbody>
</table>

Table IV: Association between secretor and non-secretor status of salivary blood groups with core gene positive and core gene negative.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Core gene positive</th>
<th>Core gene negative</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secretors</td>
<td>25 [69.4%]</td>
<td>25 [48.1%]</td>
<td>0.047</td>
</tr>
<tr>
<td>Non-secretors</td>
<td>11 [30.6%]</td>
<td>27 [51.9%]</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>52</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Hepatitis B virus strains are quite species specific, though humans remain the principal reservoir and virus is primarily found in the blood of infected individuals and other body fluids including saliva, nasopharyngeal fluids, urine, semen and menstrual fluid. Among the body fluids which become source of transmission of hepatitis B virus directly or indirectly, chances of sharing of saliva are much more as compared to other body fluids. As saliva may be shared during drinking, eating and in married couples during kissing; hence, the chances of transmission of hepatitis B virus through the saliva become greater. The current literature is suggesting that saliva is a vehicle for horizontal transmission of hepatitis B virus. However, the association between the salivary blood groups and transmission of hepatitis B has not been yet investigated. Therefore, in the present study we took one step forward to determine the salivary blood groups [secretor and non-secretor] status and transmission of hepatitis B virus.

In the present study, there was an association between salivary blood groups [secretor and non-secretor status] with core gene positive and core gene negative (p=0.047).
Although, the association is not strongly supportive, but it may provide a novel idea, and forum for further research and discussion among the researchers. In the long-run it has been known that many diseases have an association with blood groups as well as secretor and non-secretor blood group status of the subject. The secretor system offers two options: a subject can either be a secretor (Se) or a Non-secretor (Se). This is completely independent of either blood types A, B, AB, or O. Secretor blood type has antigens present in the body fluids such as saliva in mouth, mucus in digestive tract and respiratory cavities, semen etc. Vidas et al. demonstrated that patients secretor status ABO blood group antigens is possibly a factor influencing the development of high intensity of oral disease and epithelial dysplasia in non-secretor group.

Raza et al. showed the association between non-secretion of blood group antigens and respiratory virus diseases, they found that the secretion of blood group antigen is associated with respiratory virus diseases.

Emeribe and Ejezie demonstrated that blood group O donors had the highest HBsAg prevalence rate of 4.3% as against the 0% frequency for group AB donors. There was no significant association between ABO blood group distribution and the presence of HBsAg.

In addition, Behal et al. reported that the Rh-negative blood group donors (21/873) and Rh-positive group donors (429/19127) had almost equivalent prevalence rates of HBsAg. HBsAg was more prevalent in blood group B donors (174/7426) and less prevalent in AB blood group donors (38/2032). They have also reported that the HBsAg positivity was not associated with ABO blood groups.

Karayiannis et al. conducted a study on saliva, urine and seminal fluid on patients who were positive for hepatitis B surface antigen and subjects were examined for the presence of HBV-DNA. They showed that HBV-DNA was detected in saliva (88%), urine (55%), and seminal fluid (62%). Moreover, they suggested that detection of HBV-DNA in saliva, urine and seminal fluid indicates the likely presence of hepatitis B virus particles in these secretions and establishes them as potential vehicles for the infectivity.

Suwanna et al. arrived at the conclusion that the saliva of HBV carriers might be potentially infectious and that saliva testing could serve as an alternative technique for identifying HBV carriers. They also found that saliva samples of those subjects who were positive HBsAg from whom 50% were positive for HBV DNA, and those subjects who were recruited as control group were negative for HBsAg were also negative for HBV DNA.

Chen et al. examined the presence of hepatitis B surface antigen (HBsAg), hepatitis B core antigen (HBCAg), and hepatitis B virus (HBV) DNA in parotid tissues from subjects with positive serum HBV markers and demonstrated that HBV in saliva might originate from the infected salivary glands and the infectious saliva could transmit HBV.

Similarly, Kidd et al. tested the chronic HBV carriers for the presence of HBV DNA in serum, saliva, nasopharyngeal fluid, urine and tears by polymerase chain reaction (PCR) methods. They showed that HBV DNA was found in 2 urine samples, 10 saliva samples, 5 nasopharyngeal swabs and in tear fluid from 4 patients. They also demonstrated that highly viraemic HBV carriers may have high titres of HBV DNA in body fluids including saliva.

The present study was conducted based on the hypothesis that the subject, who belongs to salivary blood group antigen positive, can transmit the disease and those who are salivary blood group antigen negative can not transmit the disease. In the present study, we found that core gene positivity was significantly higher in secretor [69.4%] compared to non-secretors [30.6%].

**CONCLUSION**

There was an association between the subjects with secretor and non-secretor status of the salivary blood group antigens and core gene positive and core gene negative. In core gene positive secretors were significantly higher compared to non-secretors, although, the association is not strongly supportive but it may provide a novel idea and forum for further research and discussion. It is suggested that large sample size studies are needed to further confirm this association between salivary blood group antigens and core gene positive and core gene negative for the transmission of hepatitis B.

**Acknowledgement:** This work was supported by grant 05-492, College of Medicine Research Centre (CMRC), King Saud University, Riyadh, Saudi Arabia. We would like to express our deepest gratitude to Director CMRC and Deanship of Scientific Research, KSU, for funding this research project and their support during the study. We also thank Prof. Ashry Gad, Department of Family and Community Medicine, College of Medicine, KSU for statistical analysis. We must thank for all the patients who contributed in this study.

**REFERENCES**


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