Distribution of Haptoglobin Phenotypes among Patients with Different Types of Cancer in Sudan

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Abstract

In this study we aimed to find possible correlation between varying cancer types and haptoglobin phenotypes Hp1-1, Hp2-1 and Hp2-2. Haptoglobin (Hp) phenotypes among patients with different types of cancer in Sudan were detected by polyacrylamide gel electrophoresis followed by benzidine staining of the gels. The results indicated that individuals with Hp1-1 phenotype were significantly higher among patients with Leukaemia and patients with liver cancer when compared to the controls. Also a slight increase frequency of Hp2-2 and a decreased Hp2-1 frequency among patients with lymphoma were found when compared to the controls. And no significant difference was found in the distribution of Hp-phenotypes among patients with other types of cancer and controls. In addition, as it has been expected, no correlation was found between the distribution of Hp-phenotypes and age and sex factors in both patients and healthy control groups.

We conclude from this investigation that Hp1-1 phenotype might be associated with the susceptibility to leukaemia or liver cancer.

Keywords: cancer; haptoglobin phenotypes; Sudan

Introduction

Haptoglobin (Hp) is one of the acute phase reactant proteins that is synthesized and metabolized by the liver and represents 1.3% of total plasma proteins. Synthesis of Hp is considerably lower in fetal than in adult liver, the result of a difference in transcriptional rate [1]. Haptoglobin is also found in other human fluids like bile, blister fluids, tracheobronchial secretions, milk, cerebrospinal fluids and synovial fluids [2]. Earlier studies indicated that Hp was selectively elevated in cancers over other acute-phase reactants [3] and Hp found to be highly elevated in the tumor tissue [4].

Its main function is to bind free haemoglobin (Hb) that results from intravascular haemolysis [5], to prevent both iron loss and kidney damage during haemolysis [6]. The Hp-Hb complex, which is irreversible and stable, is sent to the liver to be metabolized. This protein is characterized by a molecular heterogeneity with three major phenotypes: the homozygous Hp1-1, Hp2-2 and the heterozygous Hp2-1 [1]. The three major Hp phenotypes: Hp1-1, Hp2-1, and Hp2-2 exhibit distinct structural and functional properties with significant biological and clinical implications [7]. Hp1-1 phenotype binds more strongly with free haemoglobin, strongly inhibits the synthesis of prostaglandin [7] and found to have greater antioxidant properties than the other phenotypes, which resulted in more efficient protection of leukocytes against oxidative DNA damage [8]. Based on Hp variable functional properties, it has been observed that Hp polymorphism has some bearing on the prevalence of many disease statuses [9]. Hp appears to be related to immune response and to autoimmune and inflammatory disorders [10]. More particularly, Hp1-1 has been found to be associated with susceptibility to: falciparum malaria and the development of severe complications [11], chronic hepatitis B [12], chronic hepatitis C [13] and liver cirrhosis [12]. While Hp2-2 phenotype, has been reported to be associated with a worse prognosis in HIV infected patients [14] and with a higher risk of mortality in patients with tuberculosis [15]. In non-infectious diseases like cancer, individuals with Hp1-1 found to be the highest among patients with breast cancer [16] and leukemia [17]. A decrease in Hp2-2 phenotypes among cases with benign and malignant neoplasm of the cervix and cervical carcinomas [16], in patients with bladder cancer [18] and among patients with pulmonary adenocarcinoma of the lung was reported [19]. On the other hand, increased Hp 2-2 phenotype frequency was found among patients with gastric cancer [20] and nasopharyngeal carcinoma [21]. Excess of Hp2-1 type has been found in patients with family history of ovarian carcinoma [22] and patients with Oesophageal cancer [18]. The Hp concentration in humans is generally stable but changes with age. Haptoglobin levels may be affected by the Hp phenotype, with circulating concentrations in the following order Hp1-1 > Hp2-1 > Hp2-2 [7, 23].

Haptoglobin level has been reported to be elevated in the blood of all patients with cancer and leukaemia [16]. Serum Hp levels found to be significantly high in Non-Hodgkin’s Lymphoma (NHL) and Hodgkin’s Disease (HD) as compared to the controls [24], associated with poor outcome for overall survival in patients with epithelial ovarian cancer [25] and recently, found to be elevated in patients with small cell lung cancer [26].

Few studies have been carried out on Haptoglobin phenotypes among cancer patients in Africa and no studies of this kind are available in Sudan. Therefore, the present study was proposed to detect the distribution of Hp phenotypes among Sudanese patients with different types of cancer to find out the association between the different Hp phenotypes and the susceptibility to different types of cancer in Sudan, if any.

2. Materials and Methods

2.1. Study Design

A simple randomly selected case-control study was applied. Patients from different areas in Sudan, from both sexes, in different age groups and with different types of cancer were included in this study after consent.

2.2. Study Area and Study Group

The Radiation and Isotopes Center (RIC) in Khartoum Hospital is the main focus in Sudan for diagnosis and treatment of cancer. Patients admitted to this center throughout the year 2002 and agreed to participate in this study were included. Volunteers’ subjects from the members of Khartoum Hospital Blood Bank and members from the Tropical Medicine Research Institute, Radiation and Isotopes Centre and National Health Laboratories who have no any type of cancer and apparently healthy were included as a control group.
2.3. Collection and Storage of Samples
Base line data and clinical information about patients under study, who are histologically diagnosed as cancer’s patients, were obtained. Heparinized vacutainer tubes were used in the collection of 1 ml venous blood samples from 415 patients with different types of cancer and 84 healthy control donors after their consent. Also there were 45 serum samples with liver cancer obtained from the Virology Department at the National Health Laboratory in Khartoum for another collaborative study on the distribution of Hp phenotypes among patients with different liver diseases (HBV, liver cirrhosis and liver cancer).

The collected blood samples were processed at the Immunology and Biotechnology Department (Tropical Medicine Research Institute-National Center for Research), Khartoum. They were centrifuged at 200 g for 10 minutes and the plasma was collected into cryotubes before being frozen at -70°C until used for further testing. All the samples were tested for their haptoglobin phenotypes by the end of the year 2002 and onset of the year 2003.

2.4. Typing of the human haptoglobin in Sudanese cancer patients and healthy controls
Normal erythrocyte haemolysate was prepared by washing red blood cells in phosphate-buffered saline (PBS) followed by lysis in distilled water.

Plasma samples were incubated with erythrocyte haemolysate and mixed with loading buffer, a few crystals of bromophenol blue dissolved in 40% sucrose solution, before being applied to the gel. Haptoglobin phenotypes were separated by discontinuous polyacrylamide gel (non-reducing) according to Davis and Ornestein method [27] and modified by Linke [28] using the Mini-V 8.10 (BRL, Life Technologies Inc, Gaithersburg, USA). The separation gel (Resolving gel) had a total concentration of 4.7% polyacrylamide, and the stacking gel 2.5%.

After completion of the run, the gel was stained for 10-15 minutes with benzidine stain (0.2 gm benzidine powder, 40 ml H₂O₂, 15 ml methanol, 5 ml glacial acetic acid and 60 μl H₂O). The gel was then washed in distilled water before drying and photography.

2.5. Data Analysis
Data collected in this study were analyzed using Statistical Package for Social Sciences (SPSS). Chi²-test was used to determine the association between the distribution of Hp phenotypes among different types of cancer and healthy control group. Charts were done using Excel software.

3. RESULTS
3.1. Haptoglobin phenotypes
Different structural features were used in the typing of haptoglobin phenotypes. Hp1-1 phenotype appears as only one thick fast migrating band closer to the free haemoglobin. Hp2-1 phenotype appears as multiple fine, slow moving bands and have the same thickness in addition to a band corresponds to the Hp1-1 band.

Hp2-2 phenotype shows a series of multiple bands, which are fine and close to each other. However, the fastest migrating band appears fainter than its preceding band.

3.2. Females with different types of cancer:
Haptoglobin phenotypes distribution among 119 females: 56 with breast cancer, 24 with ovarian cancer, 17 with endometrial cancer and 22 with cervix cancer have been detected. Hp phenotypes distribution for each group of patients was compared separately with the Hp phenotypes distribution among 39 healthy control females by using Chi-Square test (χ²) test and no significant differences were found.

3.3. Males with prostate cancer:
The Hp phenotypes distribution among 52 males with prostate cancer and 45 healthy control males have been detected. No significant difference was found between the two groups.
3.4. Patients with haematological malignancies:

a) Patients with leukaemia

Distribution of Hp phenotypes among 39 patients with leukaemia (24 males + 15 females) was compared to that of 84 healthy controls (45 males + 39 females). A significant increase in Hp 1-1 phenotype was found in the leukaemia patients group; specially among males.

b) Patients with lymphomas

The Hp phenotypes distribution among 42 patients with lymphoma (32 males+ 10 females) was compared to that of 84 healthy controls. There was slight increase in Hp 2-2 phenotypes among lymphoma patients group, although there was no significant difference found.

Figure 4: Distribution of haptoglobin phenotypes among patients with haematological malignancies and healthy controls.

3.5. Patients with cancers of the gastro-intestinal tract:

a) Upper gastro-intestinal tracts:

Hp-phenotypes distribution among 26 patients with Oesophageal cancer was studied and no significant difference was found when compared to those of the 84 healthy controls.

b) Lower gastro-intestinal tracts:

Distribution of Hp-phenotypes among 50 patients with colorectal cancer (30 males+ 20 females) and 84 healthy controls has been studied. No significant difference was found between the two groups.

Figure 5: Distribution of haptoglobin phenotypes among patients with gastro-intestinal tract cancers and healthy controls.

3.6. Liver cancer:

Hp-phenotypes distribution among 48 patients with liver cancer and 84 healthy controls was compared using χ² test. A highly significant increase in Hp 1-1 phenotype has been found among the liver cancer group.

Figure 6: Distribution of haptoglobin phenotypes among patients with liver cancer and Healthy controls

3.7. Other types of cancer:

Hp-phenotypes distribution was not significant between 14 patients with nasopharyngeal carcinoma, 30 patients with oral cancer, 14 patients with urinary cancers, 26 patients with bone and connective tissue cancers when compared separately to 84 healthy controls group.

Figure 7: Distribution of haptoglobin phenotypes among patients with other different types of cancer and healthy controls.

3.8. Distribution of Hp phenotypes according to sex and age:

The distribution of Hp phenotypes according to sex was found not significantly different in both of cancer patients group and healthy control group (p = 0.801 and p = 0.192, χ² test; respectively). Also, no significant difference was found among the different age groups for each cancer type and the healthy control group. (p = 0.632 and p = 0.317, χ² test; respectively).
Table 1: Haptoglobin phenotypes distribution and its significance among patients with different types of cancer and different healthy control groups:

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>Haptoglobin-phenotypes</th>
<th>Total number of cases</th>
<th>Hp-phenotypes Cases Vs controls ($\chi^2$-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hpl-1</td>
<td>Hpl-2</td>
<td>Hpl-2</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>15 (26.8%)</td>
<td>26 (46.4%)</td>
<td>15 (26.8%)</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>6 (25.0%)</td>
<td>10 (41.7%)</td>
<td>8 (33.3%)</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>3 (17.6%)</td>
<td>9 (52.9%)</td>
<td>5 (29.4%)</td>
</tr>
<tr>
<td>Cervix</td>
<td>6 (27.3%)</td>
<td>8 (36.4%)</td>
<td>8 (36.4%)</td>
</tr>
<tr>
<td>Healthy control females</td>
<td>5 (12.8%)</td>
<td>19 (48.7%)</td>
<td>15 (38.5%)</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>12 (23.1%)</td>
<td>27 (51.9%)</td>
<td>13 (25.0%)</td>
</tr>
<tr>
<td>Healthy control males</td>
<td>12 (26.7%)</td>
<td>22 (48.9%)</td>
<td>11 (24.4%)</td>
</tr>
<tr>
<td>Leukaemia</td>
<td>20 (51.3%)</td>
<td>12 (30.8%)</td>
<td>7 (17.9%)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>9 (21.4%)</td>
<td>15 (35.7%)</td>
<td>18 (42.9%)</td>
</tr>
<tr>
<td>Oesophageal cancer</td>
<td>7 (26.9%)</td>
<td>11 (42.3%)</td>
<td>8 (30.8 %)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>10 (20.0%)</td>
<td>24 (48.0%)</td>
<td>16 (32.0%)</td>
</tr>
<tr>
<td>Liver cancer</td>
<td>24 (50.0%)</td>
<td>14 (29.2%)</td>
<td>10 (20.8%)</td>
</tr>
<tr>
<td>Nasopharyngeal cancer</td>
<td>3 (21.4%)</td>
<td>7 (50.0%)</td>
<td>4 (28.6%)</td>
</tr>
<tr>
<td>Oral cancer</td>
<td>8 (26.7%)</td>
<td>12 (40.0%)</td>
<td>10 (33.3%)</td>
</tr>
<tr>
<td>Urinary cancers</td>
<td>1 (0.7%)</td>
<td>8 (57.1%)</td>
<td>5 (35.7%)</td>
</tr>
<tr>
<td>Bone and connective tissue cancers</td>
<td>3 (11.5%)</td>
<td>15 (57.7%)</td>
<td>8 (30.8%)</td>
</tr>
<tr>
<td>Healthy controls (males + females)</td>
<td>17 (20.2%)</td>
<td>41 (48.8%)</td>
<td>26 (31.0%)</td>
</tr>
</tbody>
</table>

* Highly Significant difference

4. DISCUSSION

In this study the distribution of Hp phenotypes among Sudanese patients with different types of cancer was detected. Among the healthy control group the frequency of Hp2-1 phenotype was the highest (48.8%) followed by Hp2-2 (31.0%) and Hp1-1 (20.2%). Similar results have been reported earlier in Sudan [11 and 29]. While among the group of patients with different types of cancer various patterns of Hp phenotypes distribution were found. The present results suggest no association between Hp phenotypes distribution and the occurrence of breast cancer. Similar results were reported by [9, 30 and 31]. Furthermore, it has been mentioned that in patients with familial breast cancer, the frequency of Hp1-1 and Hp2-1 was higher and Hp2-2 was lower than that in non-familial and control group [9]. However, earlier studies have mentioned over-represented Hp1-1 phenotypes among patients with breast cancer [16]. Furthermore, there was no association found between Hp phenotypes and susceptibility to different female’s gynaecological cancers (cancers of the ovary, cervix, and endometrium) in Sudan. While in Sweden; a significant increase in Hp2-1 among patients with family history of ovarian carcinoma was reported [9, 31]. Further investigations on a larger number of females with breast cancer and with gynaecological tumors are needed in Sudan to confirm the present findings.

In males with prostate cancer, no association was found between Hp phenotypes and susceptibility to prostate cancer and this coincides with the findings of [38]. In haematological malignancies; a highly significant increase in Hp1-1 phenotype (51.3%) was found among patients with leukaemia and this finding coincides with that of [17]. However, in 188 Brazilian patients with leukaemia a high incidence of undetectable Hp (Hp0) and the highest incidence of the HP2 gene among 48 patients with chronic lymphatic lukaemia (CLL) were reported [32]. The Hp0 phenotype may be primarily due to either deletions that remove the HP genes or to point mutations that inactivate them [33, 34]. These data must be carefully evaluated because patients with leukaemia often have associated conditions that can reduce the plasmonic Hp levels, such as hemolysis or hepatic disease [32]. Therefore, we conclude that Sudanese individuals with Hp1-1 phenotype might be more susceptible to leukaemia than individuals with other Hp types. While no association found between Hp phenotypes and susceptibility to lymphoma and this is similar to the findings in India [24].

Also, no associations were found between Hp phenotypes and susceptibility to oesophageal or colorectal cancers. However, in a similar study in India a significantly increased frequency of Hp 2-1 (59.7%) was reported in patients with oesophageal cancer [20]. On the other hand, a highly significant increase in Hp1-1 phenotypes was found among patients with liver cancer when compared to the healthy controls. No other studies were found on Hp-phenotypes and liver cancer. However, other studies on different liver diseases reported the same findings. A significant increase of Hp1-1 frequency has been reported in patients with hepatitis B [12] patients with chronic hepatitis C [13] and in patients with liver cirrhosis [12]. Therefore, it is reasonable to conclude that individuals with Hp1-1 phenotype might be more susceptible to different types of liver diseases including liver cancer than individuals with other Hp phenotypes.

For the other types of cancer; no correlations were found between susceptibility to each type and the distribution of Hp-phenotypes. In France, no correlation was found between the distribution of Hp types and patients with transitional cell cancer of the bladder [39]; while in Germany, a statistically significant decrease of Hp 2-2 phenotype was found in patients with bladder cancer [18]. And no correlation was found between the distribution of Hp phenotypes and renal adenocarcinoma [40]. In this study when Hp phenotypes distribution was detected according to gender, and age factors the statistics did not support any difference in both of the cancer patients group and the healthy control group. Such finding was expected because Hp gene is located on chromosome 16q22 and is inherited as a somatic characteristic [1]. This might be supported by the finding of [41] who reported that Hp levels fall significantly during the first decade of life for both males and females and climbed thereafter and both sexes displayed a similar pattern.

The biologic functions of Hp can be related to its ability to bind haemoglobin or to modulate immune response [42]. It acts as an antioxidant, has antibacterial activity and plays a role in modulating many aspects of the acute phase response [43]. Also, Hp reported to be involved in the breakdown of gelatin and facilitates cell migration through accumulation of a temporary gelatin matrix [44]. It is highly expressed in arterial tissue and is involved in arterial restructuring.
and expressed also in oncological tissues. This function may also be applied to other functional and pathological restructuring processes such as angiogenesis, tissue repair, and tumor cell invasion [45]. In earlier studies angiogenesis has been mentioned to play an important role in a variety of physiological and pathological conditions, including tumor growth, wound healing, and chronic inflammation diseases. Hp has been identified as one of the serum angiogenic factors that is required for proliferation and differentiation of endothelial cells in the formation of new blood vessels [46]. Hp has been found to be produced at a high level in the tumor tissue [4]. Probably the most important biological function of Hp consists in the host defense responses to infection and inflammation, acting as a natural antagonist for receptor-ligand activation of the immune system. Therefore, Hp immunomodulatory effects and Hp levels should be investigated to be used as a marker to assess the susceptibility to different types of cancer, and to assess the success of treatment and the recurrence of the disease. Also, base-line data on Hp phenotypes and levels in relation to different ethnic groups is important information to monitor the malignant diseases. Furthermore, Hp phenotypes and levels/ each cancer type for each susceptible organ, in familial and non-familial types of cancer need to be fully investigated in Sudan.

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6. Authors' Information
Ibrahim NE assisted in designing the experiments, performed the entire work and wrote the manuscript; Osman OF is the co-supervisor and assisted in text revision; Konyoz EH assisted in discussion and revised the text; Ahmed HM helped in the samples collection; Elagib AA helped with experimental design and supervised the work.

References

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