A Comparative Study of Serum Levels of Total Sialic Acid and Hanganutziu and Deicher (HD) Antibody in Normals and Cancer Patients

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Summary

An area of great hope in clinical tumor management is the development of a tumor marker that will enhance early diagnosis, assessment of prognosis and response to therapy. A tumor marker is an indicator for the presence of a tumor e.g. a biochemical molecule that can be detected in plasma, other body fluids or tissue. Tumor marker levels show correlation with disease stage, response to therapy, extent of disease and tumor burden. Elevated marker levels would be seen in patients with small amounts of tumor present, patients with advanced disease and those not responding to therapy. Blood sialic acid and HD antibody may be regarded as useful markers for a variety of cancers and could be reliable in predicting early malignant change, local progression and metastases during both therapy and follow-up.

N-acetylneuraminic acid (Neu5Ac) is a sialic acid normally present in human cell membranes. However, several human cancers are known to express N-glycolylneuraminic (Neu5Gc) acid sialoglycoconjugates, absent from normal human tissues and fluids. Such Neu5Gc-containing sialoglycoconjugates are called **Hanganutziu and Deicher (HD) antigens**. Cancer patients generate high titre antibodies against HD antigens known as **HD antibodies**.

The measurement of sialic acid levels has been shown to be a useful tumor marker. However, elevated levels of sialic acid have been detected in other pathological conditions hence measurement of HD antibodies in blood samples could be a more reliable method for tumor management. The sensitivity of HD antibody measurements can be improved by detecting antigen-bound antibody after dissociating the immune-complexes.

This study addressed the issue of the presence of a pathological level of the marker as a biochemical indicator accompanying a neoplastic process versus its absence in health. The relationship of the marker to age, sex, treatment and response to therapy was also assessed. The study therefore evaluated the usefulness of both sialic acid and HD

antibodies in the diagnosis of cancer, their possible role in determining response to therapy and as an indicator of relapse during the follow-up period.

Levels of total sialic acid, native HD antibody and antigen-bound antibody in 420 cancer patients undergoing follow-up at Kenyatta National Hospital were compared to those of 509 from clinically health blood donors.

The mean age of patients and controls was 36 and 37 years respectively and the range, 13-87 and 17-67 years respectively. 110 (38 %) patients were males while 182 (62 %) were females. 54 % (227/420) of patients studied had carcinoma, 12.1 % (51/420) sarcoma, 16.4 % (69/420) lymphoma, and 17.4 % (73/420) had other types of tumors. 45.1 % of patients in the age-group 15-25 years had lymphomas compared to other tumor types in the same age group. Patients above 56 years were observed to suffer from carcinoma (77 %), while there were very few cases of Sarcoma (3 %) and lymphoma (9 %) above this age. The highest proportion of patients (25.7 %) was between 26-35 years. 78 % of 227 patients with carcinoma were women while 66.7 % of 51 sarcoma patients and 62.2 % of 69 lymphoma patients were men. 68 % of the patients were receiving treatment, comprising of: 54.8 % as cytotoxic drugs, 2.1% as radiotherapy and the rest a combination of the treatment methods. Out of 173 patients examined for hemoglobin levels, 45.1 % had levels below 10.5 g/dl while 54.9 % had Hb levels above 10.5 g/dl. 16.8 % of the patients had proven infections of TB, HIV. Only 103 males and 143 females' patient samples had age and sex-matched controls.

In clinically healthy controls, TSA ranged between 0.51 mg/ml and 1.36 mg/ml with a mean of 0.82 mg/ml +/-0.03. The range in cancer patients was 0.48-1.72 mg/ml with a mean of 0.86 mg/ml +/-0.03. However, sialic acid levels were observed to increase with advanced age in study groups. The sialic acid level was 0.82 mg/ml and 0.83 mg/ml in female and male controls respectively while it was 0.87 mg/ml in the two gender groups in patients. Sarcoma had highest sialic acid level of 0.93 mg/ml compared to 0.85 mg/ml in Carcinoma and 0.81 mg/ml in Lymphoma. Mean levels in anemic patients were 0.89 mg/ml compared to 0.87 mg/ml in patients who had normal hemoglobin levels. In

patients with infections the level was 0.93 mg/ml while it was 0.86 mg/ml in patients with no infections (difference not significant p= 0.575). Sialic acid level was significantly elevated in cancer patients compared to controls (Student's *t*-test
t=3.25   p = 0.001).

The mean HA titre in controls was 16.8 and a titre of 67.4 was observed in patients. Carcinoma had the highest mean titre of 81 compared to 54 for sarcoma and 52 for lymphoma. Female patients had higher HA titres than males (a titre of 75 compared to 56) while there was no significant difference in the mean levels in the control group. In 73 anemic patients, the mean HA titre was 92 and 60 in 68 patients whose hemoglobin levels were normal (mean difference significantly high p=0.015). HA titre levels were significantly increased in patients compared to the control group (p=0.000).

In controls, HD antibody mean level was −0.016 compared to 0.004 (optical density at 405 nm) in patients. The mean levels were higher in females than males in test cases compared to controls who were negative for HD antibodies. Carcinoma had the highest mean HD antibody levels of 0.012 compared to 0.010 in sarcoma and −0.01 in Lymphoma. Patients at the age between 26-35 years showed higher mean levels of HD antibodies of 0.020 compared to other age groups. There was no significant mean difference in age groups in the controls. HD antibody level in anemic patients was 0.006 while it was 0.002 in patients who had normal hemoglobin levels (no statistical difference in the two means p=0.689). There was no significant correlation between ELISA titres of patients and sialic acid (Spearman's correlation coefficient, r = -0. 36 and p = 0.565). The level of HD antibody was significantly elevated in patients compared to age-matched controls (Student's *t*-test *t* =2.613 and p=0.009).

The immune-complex (IC) dissociated HD antibody mean level in the control group was -0.06 compared to 0.014 in test cases. These levels were significantly elevated in patients (Student's *t*-test *t*=4.948 p= 0.000). The levels of the dissociated HD antibody were independent of gender as there was no difference in the mean levels in the two gender groups (p=0.984). The levels were highest in Sarcoma compared to other types of tumors

that had negative values. There was a slight positive correlation between the native HD antibody and the IC dissociated HD antibody (Spearman's correlation coefficient r=0.067) but the relationship was not significant (p=0.272). There was marked variation in the increase of antibody titres following immune-complex dissociation observed in 40 % of the samples.

In conclusion, there is a significant difference in the circulating levels of sialic acid, HD antibody and immune-complex dissociated HD antibody in cancer patients and clinically healthy controls. The levels were significantly elevated in patients.

Sialic acid levels increased with advancing age and were independent of gender type in both study groups. The circulating levels were also increased in all cancer types.

Expression of HD antibodies in cancer disease is dependent on presence of HD antigen and has no relationship with gender type or age of patient. The sensitivity of HD antibody cannot be improved by detecting antigen-bound antibody.

As the levels of HD antibody were not dependent on any of the factors investigated, it would be more reliable in confirming diagnosis or extent of disease than sialic acid and would be a better indicator of the disease. The above results suggest that combined evaluation of the markers does not improve the value of individual marker, as there was minimal correlation between any of the above markers.