Survey of Hanganutziu and Deicher (HD) Antibody in Cancer Patients Attending Kenyatta National Hospital

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Abstract—The sensitivity of HD antibody in cancer diagnosis/prognosis could be improved by detection of Immune Complex (IC) dissociated antibody. Combined evaluation of native HD and Immune Complex (IC) dissociated antibody was carried out. Presence and titre of these antibodies in cancer patients was investigated in serum samples obtained from 420 patients with various types of tumors. Results were compared with those of 246 age and sex-matched controls. The serum samples were analysed for hemagglutination antibodies by hemagglutination (HA) test and the antibodies quantified by ELISA. Dissociation was achieved by treating the samples with Glycine Hydrochloride (pH 1.8), then neutralised by Tris-HCl (pH 7.4). Mean HA titre was 16.8 in controls and 67.4 in patients ($p<0.001$). Patients aged between 26-35 years had the highest mean titre of 75.9 ($p=0.397$) while controls of the same age had the highest mean titres of 19.9 ($p=0.043$). Carcinomas had a mean titre of 81 compared to 54 for sarcoma and 52 for lymphoma ($p=0.117$) among histological types. Female patients had a titre of 75.2 compared to 55.7 of males ($p<0.05$) while the difference by gender in controls was 15.1 for males and 19.3 for females ($p=0.199$). The mean level of native HD antibody was -0.011 in controls compared to 0.004 in patients ($p=0.03$). The levels were significantly high in carcinoma ($p=0.017$) compared to sarcoma and carcinoma type of malignancy. There was no association between HD antibody levels and age. Mean levels were higher in females than males in both study groups ($p=0.628$) ($p=0.601$). IC dissociated antibody mean level was -0.06 in the control group compared to 0.014 in test cases ($p=0.000$). Levels were independent of gender ($p=0.984$) while they were highest in sarcoma type compared to other types of tumors that were negative for the antibodies ($p=0.413$). Both native and antigen-bound HD antibodies are significantly increased in cancer disease.

Key Words: immune complex, HD antibody, ELISA

Introduction

The HD antigen is heterophilic with N-glycolylneuraminic acid as the terminal carbohydrate (Koda *et al.*, 1994). This antigen is absent from normal tissues and fluids of humans and chicken but expressed in human neoplasms (Kawai *et al.*, 1991). It is this antigen against which HD antibodies are raised. Cancer patients are known to possess HD antibodies (Mukuria *et al.*, 1986, Higashihara *et al.*, 1991). Since HD antibody is elevated in malignancy, its measurement could be a more reliable method to monitor the immunology of cancer. The presence of circulating immune complexes (CICs) is thought to complicate detection of native HD and thus dissociation of CICs could improve the sensitivity of HD antibody detection. Combined evaluation of these antibodies could improve the value of the marker.

Materials and Methods

Normal human sera

Sera from 509 blood donors considered as clinically healthy individuals were used as controls and were obtained from Nairobi Hospital Blood Bank (Courtesy of Prof. A Nyong'o, now deceased).

Cancer patients' sera

Sera from individuals diagnosed with various types of tumors were obtained from outpatients attending the Haematology and Oncology Outpatients' Clinic or from in-patients hospitalized at Kenyatta National Hospital between January 1999 and December 1999.

ELISA Procedure

This was done according to Mukuria *et al.*, (1985) using sterile microtitre plate with 96 flat-bottomed wells (Nunclon Denmark). 50ml of chloroform: methanol (2:1) solution containing 25 mg of antigen (HD3) and 25 mg of sodium deoxycholate was dispensed into each well. For the assay of each serum sample, 2 wells were coated with antigen while the other two wells were coated with sodium taurodeoxycholate. The plate was left to dry overnight at 37°C. 200 ml of PBS-containing 1 % egg albumin was dispensed into each well and the plate incubated for 1 h at 37°C. The plate was then washed thrice with 200 ml per well of 0.05 % Tween 20 in PBS before 50 ml of serum diluted ten times with 1 % egg-albumin in PBS was added. The first immunoreaction was allowed for 1 h at 37°C. Washing was repeated similarly and 50 ml of 2000-fold diluted horseradish peroxidase-labelled goat anti-human (affinity purified) IgA+IgG+IgM (heavy and light) Fab fragment was added to each well. The second immunoreaction was performed for 1 h at 37°C. Subsequent washings were repeated before 100 ml of 0.06 g/l in a glycine buffer of 2,2'-azino-di [3-Ethyl-benzathiozoline sulfonate (6)] (ABTS) containing 0.02 % hydrogen peroxide in a citric acid buffer was discharged into each well. The enzyme reaction was allowed for 1 h at 37°C after which the product was directly determined by measuring the absorbance at 405 nm using an ELISA reader (Labsystems Ltd).

Dissociation of immune complexes in sera

This was performed as reported by Miles *et al.*, (1993). The dissociation was achieved by treating the samples with a standard dissociation solution of 1.5 M Glycine Hydrochloride (pH 1.8). In a 0.5 ml microcentrifuge tube, a mixture of 70 ml of this solution and 70 ml of the serum to be tested was incubated at 37°C for 90 min to allow dissociation of immune complexes. To each tube was added 70 ml of a neutralising solution of 1.5 M Tris-HCl (p H 7.4) and the mixture incubated for 2 h at 37°C. 0.1 ml of 3-fold diluted serum was diluted a further 3.5 time by addition of 0.25 ml of 1 % egg-albumin in PBS. The diluted sample was used for the assay for HD antibodies as described above.

Statistical analysis

The data obtained was grouped, screened and entered in SPSS version 7.5 for statistical analysis. It was subjected to statistical tests of significance such as Students *t*-test in order to make comparisons between study objects and the controls, while differences in various means was by Analysis of Variance (ANOVA).

Results

HD antibody levels

In controls, the HD antibody mean level was -0.02 compared to 0.004 in patients (Figure 1). Carcinoma had the highest mean HD antibody level of 0.012 compared to 0.01in sarcoma and -0.01 in lymphomas as shown in Table 1. The mean level was higher in females than males in test cases compared to controls that were negative for HD antibody (Table 2).

The range of ELISA values was between -0.59-0.328 in controls with 75 % of samples with values of up to 0.02 while patients had a range between -0.179-0.198 with 75 % of patients having values of up to 0.03.

Patients at the age between 35-66 years showed higher levels of 0.02 compared to other age groups as shown in Table 3. There was no significant mean difference within age groups in controls.

Patients of the age between 26-35 years showed the highest mean levels of HD antibody of 0.019. Levels of HD antibody were significantly elevated in patients compared to age-matched controls (Student's t-test t=2.613, p=0.009).

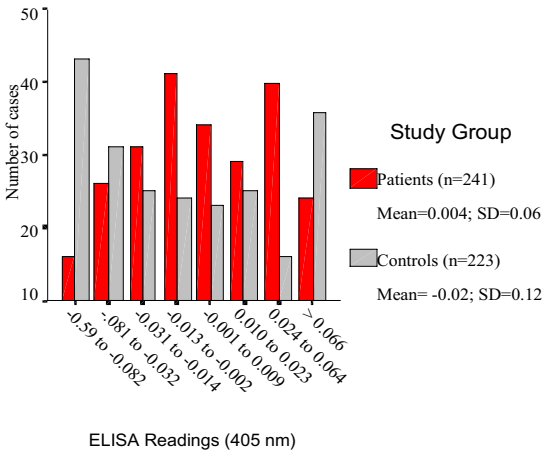

Figure 1: Elisa readings for HD antibody for 235 patients and 204 controls

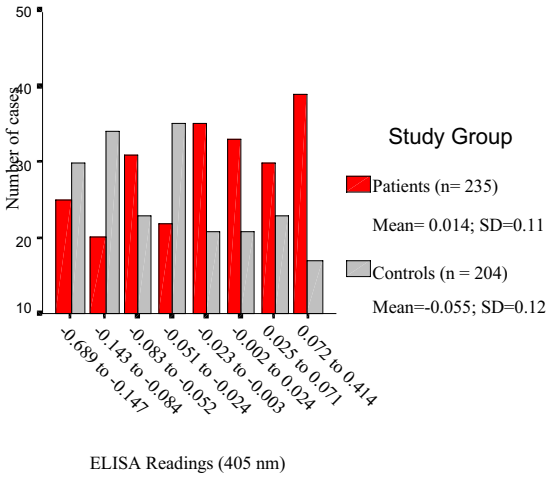

Figure 2: Elisa readings for IC dissociated HD antibody for patients and controls

Table 1: HD antibody values for different tumor types

Cancer Type	Mean	(SD)
Carcinomas	0.01	(0.05)
Sarcomas	0.01	(0.05)
Lymphomas	-0.01	(0.05)
Others	-0.01	(0.05)

Statistical mean difference by ANOVA was p=0.017

Table 2: Values of HD antibody of the gender groups

	Controls		Tests	
Gender Group	No.	Mean	No.	Mean
Female	91	-0.02	139	0.01
Male	132	-0.01	102	-0.00

Statistical mean difference in the two gender group by ANOVA was p=0.63

Table 3: HD antibody levels by age

	Tests		Controls	
Age Group	Mean	(SD)	Mean	(SD)
15-25	-0.012	(0.05)	-0.02	(0.08)
26-35	0.019	(0.06)	0.00	(0.11)
36-45	-0.014	(0.05)	-0.03	(0.1)
46-55	0.004	(0.06)	0.00	(0.11)
56+	0.005	(0.03)	-0.02	(0.09)

IC dissociated HD antibody

IC dissociated HD antibody mean level in the control group was -0.055 compared to 0.014 in test cases (p=0.000) as shown in Figure 2.

The dissociation was achieved by use of Glycine hydrochloride (pH 1.8) then neutralised by Tris-HCl buffer (pH 7.4). The dissociated antibody was quantified by ELISA. The range was -0.68-0.20 in controls and -0.49-0.414 in patients.

Dissociated HD antibody value was 0.017 in sarcomas while other histological types were negative for HD antibody (Table 4).

As shown in Table 5, male patients had a level of -0.009 while controls had -0.068. Female patients had a value of -0.018 and -0.035 in controls. The values were independent of gender (p=0.984).

The overall mean value for both gender types in both study groups was negative for HD antibody.Mean value was highest at the age bracket of 46-55 years in patient cases (Table 6).

Table 4: IC dissociated antibody levels in different cancer Types

Cancer Type	Mean	(SD)
Carcinomas	-0.02	(0.1)
Sarcomas	0.02	(0.1)
Lymphomas	-0.02	(0.13)
Others	-0.01	(0.13)

Only sarcomas were positive for IC dissociated HD antibody.

Table 5: Immune complex dissociated antibody values by gender

	Controls		Tests	
Gender Group	No.	Mean	No.	Mean
Female	81	-0.04	138	-0.02
Male	123	-0.07	102	-0.01

Table 6: IC dissociated antibody levels by age

	Tests		Controls	
Age Group	Mean	(SD)	Mean	(SD)
15-25	-0.02	(0.14)	-0.03	(0.11)
26-35	-0.01	(0.09)	-0.08	(0.14)
36-45	-0.03	(0.10)	-0.06	(0.12)
46-55	0.01	(0.14)	-0.05	(0.11)
56+	-0.01	(0.08)	-0.02	(0.13)

Only age group 46-55 years was positive for dissociated antibody.

Discussion

Levels of HD antibody and IC dissociated antibody were significantly elevated in patients compared to healthy subjects. Presence of native HD antibody in serum of cancer patients was shown as indicated by positive ELISA values. This is an indication that HD antibody producing cells are stimulated in many patients following expression of HD-antigen active molecules. HD antibodies were detected in two histological types of tumors namely carcinoma and sarcoma, while lymphomas were negative for HD antibody.

Female patients had higher levels compared to males. This difference could be attributed to the nature of the tumor type rather than sex difference. Only age groups 26-35 and 46-55 years were positive for HD antibody. This could be indicative of disease presence since majorities of patients were within this age bracket. In this study, 40 % of samples were positive for IC dissociated antibody following disruption of immune complexes. Miles *et al., 1993* have used dissociation of immune complexes to improve on serological detection of HIV infection in neonates.

The presence of CICs in this study was thought to complicate detection of native HD antibody. There was a slight positive correlation (r=0.067) following dissociation though the association with levels of native antibody (ELISA titre) was not significant (p=0.272). The results indicate that there is a great variation in the increase of antibodies following dissociation. There was a statistical significant increase in IC dissociated antibody titres in patients compared to clinically healthy controls (p=0.000). Earlier studies by Vlock *et al.*, 1993 had reported correlation of dissociated antibody with the level of differentiation of squamous cell carcinoma of head and neck and indeed observed marker variation in the increase of antibody titres.

Conclusion

Both native and IC dissociated HD antibodies are significantly increased in malignancy.
As there was no correlation between any of the above forms of HD antibody, combined evaluation of the markers does not seem to improve the value of individual marker.

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