"Detection of heterophile antibodies in humans of different age group from a teritary care Hospital."

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ABSTRACT:

Introduction: Historically heterophile antibodies have been sheep agglutinins associated with infectious mononucleosis. Present study was planned to detect heterophile antibody of different age group in humans by various animals RBC.

Methods: Serum samples were collected from people of different age group, blood is allowed to clot, centrifuged, serum separated and preserved in vials. Blood is collected from rabbit, guinea pig, sheep, human and fowl. From rabbit and guinea pig blood is collected by cadiac puncture, sheep blood from jugular vein, fowl blood from the wing vein, human blood from cubital vein. Test sera are diluted in microtitre plate and RBC of various animals were added and incubated for 1 hour and observed for agglutination.

Results: Of 500 sample, 252(50.4%) were from females and 248(49.6%) were from males. A total of 116(23.2%) sera did not show any agglutination against sheep RBC. 20 sera show agglutination at titer of 1:8. Against rabbit RBC only 11 showed titer of ≤ 1:8. Guinea pig RBC showed agglutination of ≥ 1:16 and as expected sera did not show any agglutination against fowl RBC.

Key words: *Heterophile antibody, human sera, animal blood, agglutination*

INTRODUCTION:

Historically heterophile antibodies have been sheep agglutinins associated with infectious mononucleosis. These antibodies are developed against poorly defined immunogens.These antibodies are exclusively found in patient with mononucleosis.The heterophile antibodies are not specific, as in many cases the antigen remains unknown.These antigens are important in diagnosing the heterophile antibodies in a series of diseases such as malignant tumors, lymphomas, leukemia, infectious mononucleosis, rheutamoid polyarthritis, Kawasakis disease, Mareks disease etc.

A classification of heterophile antibodies is proposed which is based on interaction with guinea pig kidney homogenate. The major group of antibodies combining with guinea pig kidney is Hangantziu-Diecher antibodies, Forssman antibodies and antibodies to New castle disease virus. Antibodies which fail to combine with guinea pig kidney are primarily those of Paul Bunnel variety.

Heterophile antibodies known as Forssman antibodies are present in sera of most normal individuals. Heterophile antibodies that are present in normal human sera play an important role in graft rejection. Heterophile antibodies persists for months and sometimes a year or more in persons who infected with infectious mononucleosis. Present study was planned to detect heterophile antibody of different age group in humans by various animals RBC.

MATERIALS AND METHODS:

Serum collection: A total of 500 serum samples were collected from people of different age group who were attending microbiology laboratory at tertiary care hospital. The blood is allowed to clot, centrifuged at 2500rpm for 5 min, serum separated and preserved in vials at -20°C. The patient's name, age and sex are noted.

Preparation of RBC as antigen suspension from different animals**:** About 5ml blood is collected from rabbit, guinea pig, human, sheep and fowl. From rabbit and guinea pig blood is

collected by cardiac puncture, sheep blood from the jugular vein, fowl blood from the wing vein, human blood from cubital vein. All the blood which is collected from each animal was preserved in EDTA cuvettes. The blood from different animals which was collected is taken in 8ml test tubes. About 2.5ml of blood is taken and then normal saline is added upto 3/4th of test tube. It is centrifuged at 3000rpm for 10 min. The procedure is repeated for 3 times. After the last wash the pack of RBC was collected and stored at 4°C.Blood once collected was used for two weeks only.25µl of washed RBC were added to 5ml of normal saline and mixed well. Now the 0.5% suspension is ready. Like this with all 5 different types of RBC suspensions were prepared and labeled.

Test procedure: The test sera were inactivated at 56°C for 30 minutes. Each sample was diluted in U bottomed microtitre plate. To 50 µl of various serum dilutions in different wells of microtitre plate, 50 µl of 0.5% washed RBC suspensions of various animals were added and plates were gently shaken and kept in the incubator at 37°C for one hour. Immediately after the incubation period readings were taken. A neat button of RBC was considered as no agglutination. A positive agglutination is indicated by a lattice mat of agglutinated cells to atleast half of the bottom of the U shaped well of the test microtitre plate.

Results: A total of 500 serum samples from people of different age group attending the outpatient department of our hospital were studied to see the presence of heterophile antibodies against RBC of rabbit, guinea pig, sheep and fowl. Out of these 500 sample, 252(50.4%) were from females and 248(49.6%) were from males. These sera were collected from all age group started from new born to 75 years. A total of 116(23.2%) sera did not show any agglutination against sheep RBC. 20 sera agglutinins at titer of 1:8, 5 sera at titers of 1:32 and 4 sera at titers of 1:64.

Against rabbit RBC only 11 sera showed a titer of ≤ 1:8. All the remaining 489(97.8%) sera had agglutinins of ≥ 1:16. 48(9.6%) of these had titers of ≥1:512. Guinea pig RBC showed agglutinins in titer of≥ 1:16 in 113(22.6%) sera and 387(77.4%) had agglutinins of ≤ 1:8.Similarly, while using fowl RBC, 457(91.4%) of the sera did not show any agglutinins.43 (8.6%) showed agglutinins at titer of 1:2 and 1:4.

DISCUSSION:

Out of these 500 sample, 252(50.4%) were from females and 248(49.6%) were from males. These sera were collected from all age group started from new born to 75 years. A total of 92(18.4%) specimen were from the age group 55-75 followed by 90(18%) from age group 21-25. A total of 261 (52.2%) sera were from males and 239(47.8%) were from females. Among females 60(12%) were from the age group 21-25 and 30(6%) from the age group of 55-75 and 16-20. (Table 1)

Agglutinins against sheep RBC: A total of 116(23.2%) sera did not show any agglutinins(≤ 1:2). A total of 200(40%) sera showed agglutinins in titre of 1:2 to 1:8. A total of 84(16.8%) specimen had agglutinins in dilutions of ≥ 1:16. These are the well studied heterophile antibodies of sheep agglutinins

Agglutinins against rabbit RBC: Surprisingly, many specimens showed agglutinins against rabbit RBC. Only 11 sera showed a titer of ≤ 1:8.All the remaining 489(97.8%) sera had agglutinins of ≥ 1:16, 48(9.6%) of these had titers of≥ 1:512. The distribution of positive sera in various age groups was even and no particular preponderance in any of the age group was noticed. Since the clinical details were not recorded, whether any association with any of the clinical condition exists cannot be detected. It looks like that no correlation may exist since the distribution was in all the age group and in both sexes. Absorption studies with guinea pig kidney suspension and ox cell stroma to see the specificity for infectious mononucleosis were not done.

Table No I Shows age and sex wise distribution:

Age group	Sex wise distribution		Total no of sera
0-5	M	8	12
	F	4	
6-10	M	7	10
	F	3	
11-15	M	7	8
	F	1	
16-20	M	17	47
	F	30	
21-25	M	30	90
	F	60	
26-30	M	25	56
	F	31	
31-35	M	22	38
	F	16	
36-40	M	22	44
	F	22	
41-45	M	13	30
	F	17	
46-50	M	26	40
	F	14	
51-55	M	22	33
	F	11	
>55	M	62	92
	F	30	

Agglutinins against fowl RBC: Fowl RBC are nucleated and hence are more suitable for coated particle agglutination reactions since they tend to settle down faster than the nonnuclated RBC of mammalian species.Further, the incidence of heterophile agglutinins against fowl RBC should be much low since they are taxonomically well apart from humans. As expected, 457(91.4%) sera did not show any agglutinins against fowl RBC. Only 43(8.6%) sera showed agglutinins that too in titres of 1:2 and 1:4.

Agglutinins against guinea pig RBC: Using guinea pig RBC, a total of 113(22.6%) sera showed agglutinins of≥ 1:16. The remaining 387(77.4%) had agglutinins of ≤ 1:8. The distribution of agglutinins was even in all age group and sex group without remarkable preponderance.

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