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Heterophile Antibodies amongst Normal University of Benin Undergraduate Students.

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Abstract:

The Paul-Bunnel presumptive test for infectious mononucleosis was used to determine the incidence of Heterophile antibodies in sera of under-graduate students of University of Benin. A high incidence (71.6%) of heterophile antibodies in sera of those subjects is reported. Significant heterophile antibody titer of 1in 80 and above was obtained in 5.9% of subjects. Female had a higher incidence of heterophile antibodies (75.9%) than males (66.7). The age groups 25-29 years had the highest incidence (78.5%) of heterophile antibodies. Serological testing of patients' blood was necessary to distinguish the clinical symptoms of infectious mononucleosis from those of other infectious diseases such as influenza, rubella and hepatitis. Our findings are of public health importance.

Key Words: Heterophile Antibodies, Serum, Benin University.

Introduction:

Infectious mononucleosis occurs predominantly on children and in young adults (1). It is characterized clinically by fever, exudates or membranous pharyngitis, generalized lymphadenopathy, splenomegaly, lymphocytosis with the appearance of the so-called Downy cells and a positive Heterophile titer (2).

Studies have shown that Epstein Barr virus is associated with infectious mononucleosis (3). Infection of the individual with the virus results in the production of protective antibody against infectious mononucleosis (4). Infectious mononucleosis is usually recognized in late adolescent and early adult life; up to 55% of British College students possess antibodies to Epstein Barr virus while a much greater incidence of antibodies (84%) to Epstein Barr virus has been observed in college students from tropical countries (5).

Serological testing of patients' blood is necessary to distinguish the clinical symptoms of infectious mononucleosis from those of other infectious diseases such as influenza, rubella and hepatitis (6). Test for heterophile antibody has remained the only serological diagnostic test for infectious mononucleosis. The detection of IgM to capsid antigen of Epstein-Barr virus of great diagnostic value in patients with infectious mononucleosis who are negative for heterophile antibody is doubtful (7).

Although the clinical disease is common in temperate and sub-tropical areas of the world it is rare in Nigeria and other African countries (8). Using the immunodiagnostic kits Adadevoh et al (8), reported a high incidence of serological positive test for infectious mononucleosis among patients of varying disease conditions in Ibadan Nigeria. This led them to suggest that the clinical manifestation of infectious mononucleosis in the environment may differ from that classical picture seen else where.

The disease is mostly recognized in young adults and infection appears to give high degree of resistance to subsequent re-infection. Since non-specific heterophile antibodies are elevated during infectious mononucleosis, this test facilitates diagnosis.

The present study was therefore conceived to determine the incidence of heterophile antibodies among undergraduate students of University of Benin, Edo State, Nigeria.

Materials and Methods:

Veronal buffered diluent was prepared by dissolving 20.3g MgCl2.6H2O and 4.4g CaCl2.2H2O in 100ml of distilled water. Stock buffered solution was made by combing the following in two liter volumetric flask - 83.0g sodium chloride, 10.2g sodium-5,5 diethyl barbiturate, 500ml distilled water, 34.6ml 1M hydrochloric acid, and 5.0 ml Veronal buffered diluent. This was mixed properly to form a uniform solution, and made up to 2 liters with distilled water and mixed thoroughly. The PH of the buffer was 7.3 as was obtained using a PH meter. Gelatin water solution was prepared by adding 1.0g of gelatin to 100ml of distilled water and brought to boil. This mixture was made up to 800ml with distilled water at room temperature, and was stored at 4.0 0C until needed for use.

Veronale buffered diluent for daily use was prepared by adding one volume of stock to four volumes of gelatin water solution and stored at 2.80C. One volume of packed sheep red cells, previously washed with cold veronal buffer, was added to forty Nine volumes of cold veronal buffer, diluent to obtain a two percent suspension.

Sheep red blood cell suspension was standardized by centrifuging 10ml sheep red blood cell suspension at 600 rpm for 5 minutes to determine the volume of the packed cell. The suspension yields 0.2ml packed cells indicating the cell suspension was 2.0%. The sheep red blood cell suspension was stored at 2-8%. The cells should be washed and re-standardized for use on subsequent days.

The study population consisted of 102 normal, male and female under-graduate students of the University of Benin. Their age ranged between 15 and 31 years. Five ml venous blood were obtained from each student, allowed to clot, and the sera separated from the clothed blood by centrifugation. The sera were stored at -200C until required for use. Before use compliments were inactivated by incubating the sera at 560C in a water bath for 30 minutes.

The test was carried out as described by Conrath (9), and Med-Ox Diagnostic Incorporated (10). A 1:10 dilution of each test serum was made by adding 0.1ml of serum to 0.9ml of veronal buffered diluent. Two fold serial dilutions were made by adding 0.05ml of test serum with a micro dropper pipette into the first six wells (made of 12 wells) of the micro titer 'V' plate. Serial dilution was made into subsequent wells

containing 0.025ml of the veronal diluent by adding 0.025ml of 2%sheep red blood cell to all the wells (test and control well),(Table1). The whole dilutions were mixed by mechanical agitation. All plates were sealed and incubated at room temperature for an hour. Agglutination was read and plates stored at 40C overnight after which they were incubated at room temperature for 15 minutes to elute cold agglutinins.

Results:

Seventy three (71.6%) of the 102 undergraduate students were positive for heterophile antibodies. Six (5.9%) had titer of 1:80 and above. Females had a higher incidence of heterophile antibodies 41(75%), than males who had 32(66.7%), (table 1). The age group with the highest positive heterophile antibody (78.6%) was 25-29 years. This was followed by age group 20-24years with (73.1%), and lastly by age group 15-19 years with (55.6%), (table 2).

Table1: Determination of Heterophile antibody titers.

Tubes/wells	1	2	3	4	5	6
Veronal buffer/Serum	0.9/0.1	0.05	0.05	0.05	0.05	0.05
Veronal buffer diluent		0.025	0.025	0.025	0.025	0.025
2% sheep Rbc		0.025	0.025	0.025	0.025	0.025
Titers		1:20	1:40	1:80	1:160	1:320

Table 2: Incidence of heterophile antibodies in the study population.

Sex	Number of Students	Number, (%) Positive
Male	48	32, (31.4)
Female	54	41, (40.2)

Table3: Age specific distribution of heterophile antibodies among studied population:

Age group in years	Number of students	Number, (%) positive titer
15-19	9	5 (4.9)
20-24	78	57 (55.9)
25-29	14	14 (13.7)
30-34	1	1 (0.9)

Discussion:

The Paul-Bunnel presumptive test for infectious mononucleosis which was used for this study, and involves agglutination of sheep red blood cells, has over the years been the traditional method of demonstrating heterophile antibodies in serum samples (11).

Sheep red blood cell agglutinations are not specific for infectious mononucleosis. They are known to occur in a number of other conditions such as serum sickness, infectious hepatitis, rubella, leukemia, and Hodgkin's disease, and low titers can also be demonstrated in the serum of some normal persons (12). In general, the agglutination titer is higher in infectious mononucleosis than in other disease conditions. There is a good evidence that a titer of over 1:80 is diagnostic of infectious mononucleosis, so that any differential absorption test is unnecessary at this stage (13).

It has been reported that students from tropical countries had higher incidence of antibodies (84%), than those from temperate countries (14). However, Adadevo, Sobulo and Ogbimi (15), using commercial serological test kits found a high percentage of antibodies to infectious mononucleosis in patients of varying diagnosis and suggested that to infectious mononucleosis may present differently in Nigeria and other African countries.

Results in this study have also shown that the highest incidence of heterophile antibodies was within the age group 25-29years but the age group that had significant antibody titer was between 20 and 24 years. Thus the age that is potentially most affected in Benin-city is between 20 and 24years which coincides with the age groups reported in developed countries, (16).

Female students had a higher incidence as well as higher titer of heterophile antibodies than male students. The reason for this observation is not immediately clear. It may be inferred from this study that institutionalization and age may be associated with the incidence of

infectious mononucleosis since both studies were done on college students. More work is therefore required to confirm this.

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