Detection of Immune complexes in serum of infected rabbits with *Trypanosoma evansi*

*Khairy A. Dawood
* Bahaa A. Latif
**Ala AL-Amran

Summary

Immune complexes detected in serun of rabbits infected by *Trypanosoma evansi*. Which are reached 8 mg/ml of serum in fourth week of infection. Glomerulonephritis occurred due to preciptation of immune complexes on basment membrane of blood capillaries and Bowman's capsule. These material appear as homogenous without histological structures by using ordinary stain and by using horseradish peroxidase stain

Introduction

Some researchers studided the relation of the immune complexes with several diseases. Gills and Hendrickes (1963) noticed the production of immune compiexes in serum of children infected with malaria . Berger *et al* (1967) added when the percent of immune complexes increased , glomerulonephritis with disturbances in blood pressure occur. In Brazil many infected patients with *Schistosoma mansoni* suffer from glomerulonephritis. Biopsy reveal, there is conjugation between immuneglobulin with complement which adhere on the wall of capillaries (Brito *et. al* (1975) ; Silva *et .al*. (1970) .Zilton *et al* (1971) studied , the pathological changes in kidneys of. Patients infected with hepatosplenic schistosmiasis and diagnosed the chronic diffuse glomerulonephritis with focal thickening of basement membrane, while Losos (1980) added the skin can be used as indicator organ for the presence of autoantibodies or immune complexes.Wright *et al* (1985) worked on experiment on dogs which are injected intravenously with bovine serum albmin, thich precipitae on basement membrane of kidneys were noticed. Hussian (1986) used immuno peroxidase method for the dignosis immune complexes, which precipitated on basement membrane.Waitumbi and Nantulya (1993) used ELISA test for diagnosis of immune complex, which are precipitated in kidneys camels infected by *Trypanosoma evansi*.In our persent work, method of kharazmi *et al* (1982) used for detection of immune complexes in serum of rabbits infected by *Trypanosoma evansi*

*College of Medicine/University of AL-Qadisyia
** Science College /University of Al-Nahren

Materlals and Mothods

Kharazmi methods

1- polyethylene glycol (30 lel) molecular, weight (20.000) mixed with 0.15 ml3 of rabbit serum in veronal buffer pH.7.6 then left in refergator 4°C for 24 hours.
2- Centrifuged 1000 R/M for min., supernatant discarded and the pricipitae washed by using 0.1 ml3 polyethelene glycol 2% which is solved in 0.01 MEDTA.
3- By using the centrifuge the precipitate solved in 0.15 ml 3 of vernal buffer.
4- Finally this solution examined for detection immune Complexes.

Preparation of Anti- Immune complexes

Method of kaye (1976) used for preparation of antimmune complexes. Emulsion prepared by mixing 2-4 mlg of immune complexes which is precipitated from the serum of rabbit with equal volume of freuna a djuvent.

Immunization

Guinea pig injected with 50 ml of the emulsion in shoulder region in days 1,14,28,42, and 50, Blood collected the heat directly 10 ml from each animal for separation of serum.

Isolation of immunoglobulin

1- 5gm of Amonium sulphate added to 20 ml of serun of Guinea pig . Left for 24 hours in room temperature.
2- Centrifuged 4000 R/M, Supernatant is discarded
3- The precipitate washed two times with 5 ml of ammonium sulfate (1.75M)
4- The precipitate solved with amall amount of distill water and put it in dialysis bag. then left in acetate buffer 12*2 hours with two times of changing the buffer.
5- The pricipitation of dialysis is contain lipoprotein, therefore to get red of this material, so centrifugation can be used .
6- Immunoglobulins are present in supernatant . Drop of Merthiolate in 4°C. quantitatively by using spectrophotometer

Histopathological sectioning

Fixed specimens in 10% formaline were dehydrated in histokinate and then embedded in wax.

Sectioning of these samples were establish by microtome and of the slides stained by haematoxylin and eosin and other stained by immunoperoxidase method.

Detection of Immune Complexes in Kidneys

This detection depend on direct method by labelling the antibodies which are precipitated in glomeruli and react against immune complexes, and by using the substrate which produce the staining of the precipitated material (Heinzman, 1981)

Finally, slides are stained by haematoxylin for one minute covered and examine by using Light microscope.

Results

Immune complexes in serum

The percent of immune complexes is increased in rabbits which are infected dy *Trypanosoma evansi* gradually until it reach the peak the 8mlg/ml in fourth week of infection while it remain standard control group on 2 mlg/ml of serum . Then the percent of immune complexes of infected group decrase until it reaches 4 mlg/ml in thenth week of infection (Figl).Increased in percent of Immune complexe at the begging of infection and then decreased after the fourth week , due to precipitation of immune complexes in tissues and organs and some of it discarded out with urine .

Immune complexes in Kidneys

Grossly the kidneys which are affected by the precipitation of immune complexes appear rough granulated surface. Fig (2). Section of affected kidneys stained by Haematoxylin and eosin stain and also stained by horseradish peroxides . These sections were examined by Light microsope and the immune complexes noticed precipitated on basement of the glomerular capillaries as a homogenous material, red-orange in color and without cellular structures . This material also present in the glomerular space. Therefore glomerulonephritis was very clear in these section . Fig (3)

Discussion

Separation of immune complexes form the blood serum can be done several methods such as Ultracryoprecipitation, polyethyleneglycol and rapid cell binding, but the polyethyleneglycol is the most common methods used for separation of immune complexes (Heaf 1969) and Tausing (1979)Karazmi (1981) used polyethylene glycol method for separation immune complex produced due to Leishmania infection in human. Davey and Busch (1969) noticed the horse radish peroxidase is the most suitable strain for diagnosis the immune complexes. Berger *et al* (1967) diagnosed the glomerulonephritic due to immune complexes produced by malara infection. Silva (1970) and Brito (1971) noticed the immune complexes precipitated on the wall of blood capillaries of glomeruli due to infection of trypanosomiasis, while Gallo *et al* (1981) write the immune complexes can be stimulated by the injection of albumine and increased its percent in serum and its precipitation in kidneys.Patailk *et al* (1993) provided the ELISA test with immunoperoxidase van be used for detection of immune complexes in kidneys of infected rabbits which are infected with *T. evansi* .In our present work the level of immune complexes in serunm of followed for 12 weeks of infection and there is variation in this level recorded.

References

1-Gills, H.and Hendrickse . G. (1963) Renal pathological changes associate with malaria. Brit. Medi. J.2,27.

2-Berger, M.B.Brich, I.M .and Cante, N.R.(1976). Immune complexes of Malaria Ann.Int. med. 67,1163.

3-Brito ,T.D.E.Amatanet, V.Hoshine ,S.and Penna, D. (1975).Glomerular involvement in human Kala-azar Amer. J.of Trop .Med.And Hyg.32,156-160.
4-Silva, L.Brito , T.Lopes, J. (1970) . Glomerulonephritis associated with Schistosomiaisis. Bull. Wlod . Hith . org. 42,907.
5-Zilton, A.Anderade,S.Anderade, G.and Moyses, S.(1971). Renal changes in patients with Hepatosplenic scistosomiasis.Am.J. med. And Hyg.vol. 20 (1):77
6-Losos, G.J(1980). Disease caused by T.evansi Areview Vet. Res. Communication .4,166.
7-Wright ,N.G.Mohammed ,N.A.Fckfrsal ,Nash, N.S. (1985).Experimental immune complexes . Glomerulonepritis, in dogs receiving cations bovine serum albumin. Res. In Vet Sci. 38,322-328.
8-Hussian,M.N.(1986) . Glomerulonephritis of sheep Aetiology, Diagnosis and morphological classification based on lights and Electron microscope and Immunofluorescence.Ph.D.Thesis ,University of Queensland Australia.
9-Waitumbi,J.Nantuya ,V(1993). Acomparison of the antigen detection EI.AZA.and parasite detection for diagnosis of T.evansi infection in camels. Vet. Parasitology,49,159.
10-Kharazmi,K.Rezal ,R.Fani, M.and Behforouz, N.(1982). Eridence in serum and C3 b and C3d on red cells of Kala-azar pationents Trans.Roy,Trop. Hyg. 76,794-798.
11-Kaya,D. (1976). Infection endocarditis. University park press International publishers in science and medicine P.217.
12-Heinzmann, D.(1981). Serological Investigations of circulating antibodies and antigens in Trypanosoma evansi infection in the rabbit and goat before and after chemotherapy. Vet. Bull. 52,7: 498.
13-Heaf,E. (1969) . Laboratory notes for medical diagnostic. First Edition published by Hoechst phatmaceuicles p. 1-10.
14-Tausing , M,J. (1979) . Process in pathology published by Blackwell p. 175.
15-Davey F.R. AND Busch, G.J. (1969) . Immunohistochemistry of glomerunoephrtis using horseradish peroxidase and flurscin labeled antibiody. Am.J.Clin. Path . 53, 531.
16-Gallo, G.R.Tersa, G.G. andLamm, M.G.(1981). Change circulation Immune complexes as a factor in glomerular membrane Localization in mice –I.clin. Inves . 67:305.
17-Pataik, K.M.Arara,N.K.kapoor, M. (1993) . Camel trypanosmiasis in Rajasta , India, Vwt. Parasitology. 49,319.

Fig . (1) shows the immune complexes in serum of rabbits

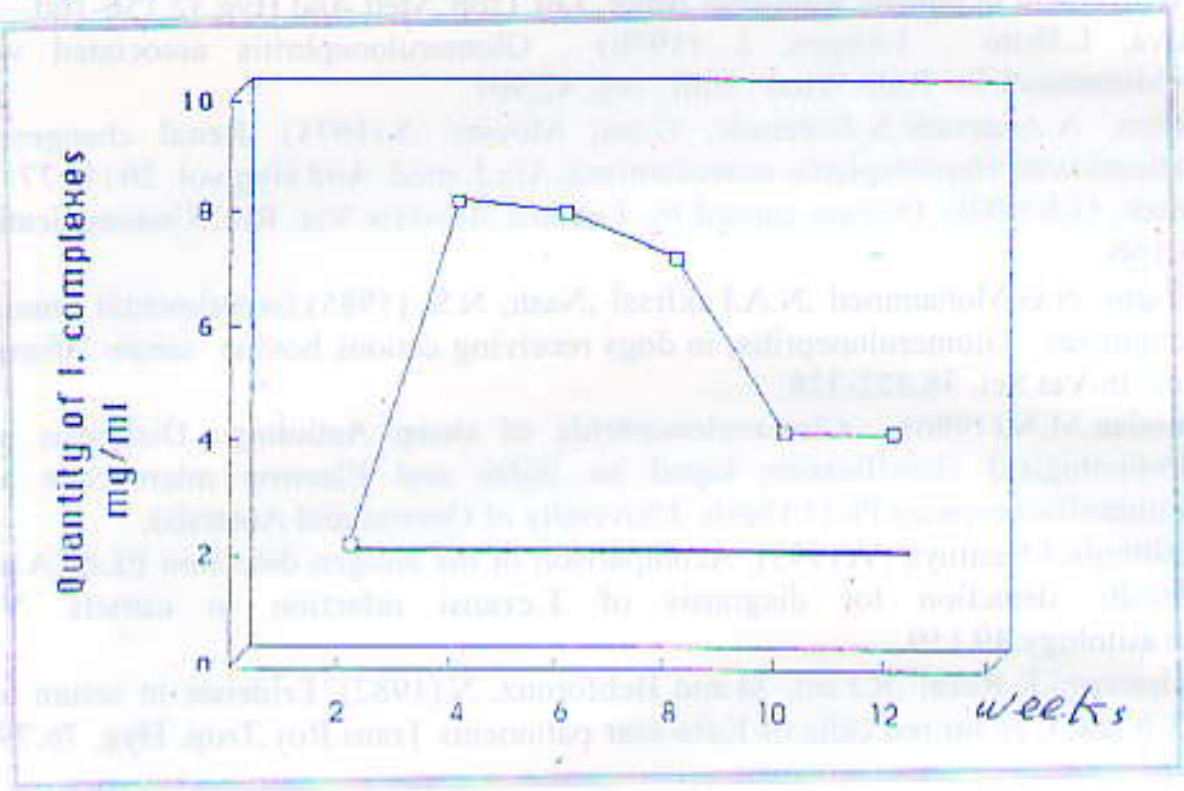

Fig (2) kidneyof rabbit in twelfth week of infection of T.evansi. Notice the granulated surfase .

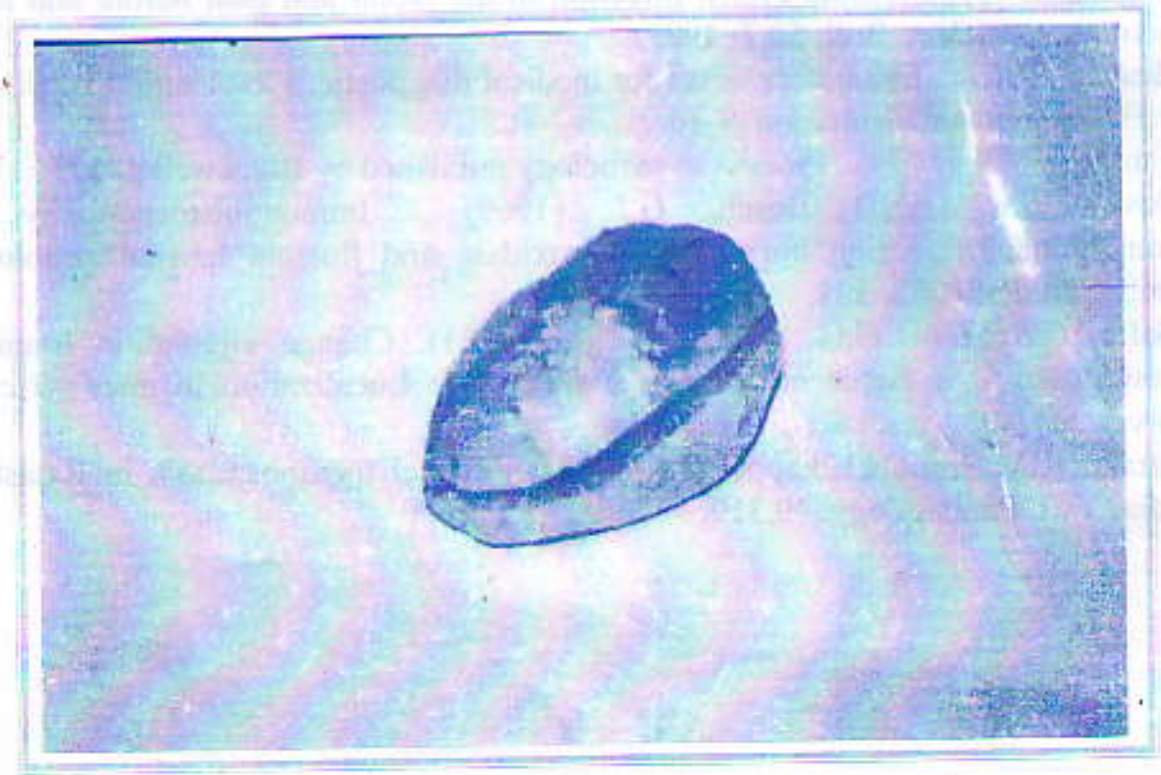

Fig (3) Glomerulus in the infected Kidney Immune complexes precipitated on the basmentmembrane of blood Vessels.