Effect of Tannic Acid on Sheep Erythrocytes in IHA Test
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Abstract:
Optimal concentration of tannic acid was found to be 0.1 to 0.4% to prepare IHA reagent for the diagnosis of human hydatidosis. Higher concentrations of tannic acid result in clumping, inhibition of sheep erythrocytes and appeared unsuitable for the preparation of IHA reagent.

Introduction:
Indirect haemagglutination (IHA) is commonly used as a quantitative serological test for the diagnosis of parasitic diseases. Imported serodiagnostic kit of hydatid disease is expensive, difficult to get every time and cannot be used for long time due to limited storage stability. Moreover, indigenous prepared IHA reagent has been found to be more sensitive than commercial reagent for the diagnosis of human hydatidosis. In order to avoid these problems, and also to exclude false negative results, it would be better to use local hydatid fluid antigen to standardize serological techniques to confirm the diagnosis of hydatid patients. In the preparation of IHA reagent, tannic acid used for tanning of sheep erythrocytes to sensitize them with specific parasitic antigens. Different concentrations of tannic acid have been used to prepare IHA reagent.

Materials and Methods:
Hydatid antigen: A crude hydatid fluid antigen was used for the preparation of IHA reagent. The hydatid fluid was collected from hydatid cysts of Echinococcus granulosus form the liver of local sheep.
Hydatid sera: Sera were obtained from three surgically confirmed hydatid patients and two albino rabbits, immunized with sheep hydatid fluid antigen were used in this study for the detection of anti-E. granulosus antibodies.

Indirect haemagglutination (IHA) test: IHA reagent was prepared initially following the process described by Krupp.

Preparation of sheep erythrocytes: Two or three-year-old sheep red blood cells (SRBCs) were washed thrice with phosphate buffer (PBS, pH 7.2).

Tanning of sheep erythrocytes: Equals the volume of 2.5% suspension of SRBCs in (PBS, pH 7.2) and different dilutions of tannic acid (0.05 to 1.0%) in PBS, pH 7.2 were mixed, and gently rotated at 4°C in an ice bath for 30 minutes.

Sensitizations of sheep erythrocytes: for sensitization equal volume of 2.5% suspension of tanned SRBCs and optimal concentration of hydatid fluid antigen (50 u.g /ml) in PBS, pH 6.4 were combined and incubated at 37°C for 30 minutes by gentle rotating.

IHA test was performed routinely in micro-titration plates, using 2.5% suspension of SRBCs in 2% normal rabbit serum (NRS)-PBS, pH 7.2.

Results:
IHA liters obtained with different concentrations of tannic acid among rabbit hyper immune serum and hydatid patients sera are shown in Table. Higher concentration of tannic acid inhibit the agglutination pattern of antigen coated sheep erythrocytes.

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Table: Effect of tannic acid for the detection of anti-hydatid antibodies.

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<th>% Concentration of tannic acid</th>
<th>Antibody Titers with</th>
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<td>Rabbit hyper immune serum</td>
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* Inhibition of agglutination of sheep erythrocytes.

Discussion:
Serological techniques are important to confirm the diagnosis of, human hydatidosis in endemic countries like Libya. Some of the advantages of preparation of indigenous IHA reagent are that the test can be performed at anytime to detect specific antibodies raised against local strain of E.granulosus in the patients. However, false positive reactions have been reported in malignant patients using hydatid fluid antigen collected from local patients. Thus, the standardization of serological techniques is important by using specific antigen specially collected from hydatid cysts from local domestic herbivorous animals.

Sheep erythrocytes have been used for a long time to prepare IHA reagent for the detection of parasitic antibodies in the serum of patients. Tannic acid is used in tanning the sheep erythrocytes to retain their ability to adsorb specific antigens.

In the present study, higher concentration of tannic acid makes it unsuitable for the proper agglutination of sheep erythrocytes in IHA test for the detection of anti-hydatid antibodies.

Therefore, to standardize IHA test, optimal concentration of tannic acid (0.1 to 0.4%) should be used to avoid inhibition of agglutination of sensitized sheep erythrocytes. Higher concentrations of tannic acid results in some spontaneous clumping and partial lysis of sheep erythrocytes.

The IHA reagent prepared from local hydatid fluid antigen, will be economical screening test and will encourage the application of the test for valuable resection of hydatid cysts and also for the control of the disease in endemic and epidemic areas.
References: