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Using of Aminothiophenol in Modified Latex Agglutination Test (MAT) for Diagnosis of Toxoplasmosis in High Risk Pregnancies in Nenava Governorate

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ABSTRACT

Using of Aminothiophenol which contain active Mercaptan group in modified Latex Agglutination Test (MAT) instead of 2-Mercaptoe-thanol for diagnosis of Toxoplasmosis in (150) pregnant women whom their age between (20≤-45≥) years, and their antibodies titer in LAT between (8-64).

The results shows that the (0.5) M of Aminothiophenol has the same efficiency with (0.2) M of 2-Mercaptoethanol compound in destroying immnoglobulin M (IgM), but the concentration (0.4) M of Aminothiophenol has low efficiency especially at titer of (64).

استخدام مركب Aminothiophenol في اختبار تلازن اللاتكس المحور لتشخيص داء المقوسات لدى الحوامل المعرضات للخطورة في محافظة نينوى

الملخص

استخدمت مادة Aminothiophenol الحاوية على مجموعة Mercaptan الفعالة في اختبار اللاتكـس المحور كبديل عن مركب 2-Mercaptoethanol في تشخيص داء المقوسات لـدى (150) امـراة حامـل واللاتي تراوحت اعمارهن بين (20≤ – 45≥) سنة، وكانت معايير الاضداد لديهن بين (8 –64) باختبـار تلازن اللاتكس.

اوضحت النتائج ان للـ Aminothiophenol بتركيز (0.5) مولاري له نفس كفاءة التركيز (0.2) مولاري من مركب 2-Mercaptoethanol في تكسير الكلوبيولين المناعي نوع M (IgM) بينمـا اظهـر التركيز (0.4) مولاري من مادة Aminothiophenol كفاءة قليلة وخصوصا عند المعيار (64).

INTRODUCTION

Toxoplasmosis is a world wide prevalent disease. Its clinical symptoms is unapparent and can not be diagnosed easily except serologically by the detection of antibodies formed against the parasite and by isolation of parasite, itself from the body fluid and different tissues (Remington et al., 2000).

The Modified latex agglutination test (MAT) is still considered as a common serological test for the diagnosis of acute Toxoplasmosis, which involves the use of 2-Mercaptoethanol (2-ME) compound as a reductive material for disulfied bounds (SH-bond) binds the five units of the immunoglobulin M (IgM). This technique makes it possible to differentiate between acute or chronic types of infection (Desmonts and Remington, 1980).

Oksanen and others (1998) indicated that it is possible to use other substances instead of (2-ME) which have a similar chemical structure like Dithiothreitol substance.

The Aminothiophenol substance also resemble the (2-ME) compound in its chemical structure where it contains the active mercaptan group which has the ability to reduce the disulfied bonds that bind the five unit of the immunoglobulin M IgM (Schmid, 1996).

This substance (Aminothiophenol) has the following chemical structure: 2-mercaptoalanine; 2-Aminophenyl mercaptan; 2-Aminobenz-enethiol and its Molecular formula is $NH_2C_6H_4SHC_6H_7NS$, it's molecular weight is (125.19), the density (1.17) gm/cm^3, boiling point is (70-72)°c, and melting point is (16-20) °c, this substance is liquid in nature and it is yellow in colour and freezes at 4°c, if stored in the room temperature in a sterile bottle (Cary, 1996).

In the present study the Aminothiophenol substance has been used instead of (2-ME) compound in modified latex agglutination test for diagnosis of active acute cases of Toxoplasmosis which need treatment especially during the first trimester of pregnancy to differential it from chronic cases of this disease this will decrease the cost of treatment in this especially under the present embargo imposed on our country.

MATERAIL AND METHODS

The preparation 1 M of aminothiophenol solution:

The aminothiophenol solution which prepared in a molarity (*1*) Molary according to this law:

$$Molarity = \frac{Weight}{Molecular\ weight}\ x\ \frac{1000}{volume\ prepared\ in\ cm^3}$$

$l = \frac{weight}{125.19}\ x\ \frac{1000}{100} = 12.519\ gm$ and its completed to (100) cm^3 by P.B.S. solution.

Because this substance is liquid, therefore we follow the law of density to reach the exact volume

$$Density = \frac{mass}{Volume}\ x\ \ percentage\ of\ the\ purified\ substance$$

Where the percentage of the purified substance is 98%,

Therefore, the $Volume = \frac{12.519}{1.17x0.98} = 10.486 \quad cm^3$

10.486 cm^3 of this substance will be taken and completed to (100) cm^3 by P.B.S. and this solution is considered as the stock solution with morality (1) M.

Preparation of the solutions Aminothiophenol substance:

In a molarity of (0.1, 0.2, 0.3, 0.4, 0.5) M solutions of different molarity have been prepared from stock solution of the Aminothiophenol substance where solution in a molarity of (0.1, 0.2, 0.3, 0.4, 0.5) M has been prepared in a volume of (10) cm^3 following this law:

$V_1 \times M_1 = V_2 \times M_2$

Where V_1: the volume of stock solution

M_1 :it's molarity

V_2 : prepared volume

M_2: it's molarity

$V_1 \times 1 = 10 \times 0.1$

$V_1 = (1)\ cm^3$

1 cm^3 of solution whose molarity is (1) M has been taken and the volume completed by P.B.S. to reach (10) cm^3. The prepared solution with a molarity of (1) M was kept in sterilized glass bottle. Following the same law the other concentration of this solution was also prepared from the stock solution. After finishing the preparation of the exact concentration, the bottles were shaken well and left for (1) hour in order to homogenize the solution, after then the solutions were filtered using medical gauze to remove undissolved sediments, the supernatant was used in each concentration instead of 2-ME compound in MAT.

Examination of serum samples by MAT:

150 serum samples has been collected from a high risk pregnant women attending Saddam General Hospital, Central Health Laboratory and Private Laboratory in Neneva Governorate. The seropositivity was examined using a kit called Toxo, Cell-latex produced from Biokit-SA Spanish Company. The positive serum samples have been taken (ie: infection with Toxoplasmosis) and the modified latex agglutination test was done twice, the first using (2-ME) compound in a concentration of (0.2)M, and the second by using the Aminothiophenol solution in a concentration of (0.5)M by mixing equal volumes of the tested serum examined and the reduced compound.

The mixture was then incubated at (37)c for one hour, after incubation a duplicated dilutions of the serum were prepared and an equal volume of the diluted serum and latex antigen (Toxo cell-Ag) were mixed, the mixture was mixed well with continous shaking for (5) minutes and the agglutination was then observed by naked eye or under Microscope using low power magnification lens (lox) and the antibody titer for every sample was recorded.

The steps of the test were repeated as mentioned aboved by using other concentration from (0.1-0.4) M of Aminothiophenol substance.

RESULTS AND DISSCUTION

Table (1) shows that the rate of acute infection among (150) serum samples taken from pregnant women at risk who were seropositive in LAT, was (64%) by using of 2-ME compound and (0.5)M of Aminothiophenol substance, while the rate of acute infection by MAT was lower than (42.60%) by using (0.4) M of this substance.

Table 1: Type of infection among pregnant women by using Modified Latex Agglutinagtion Test (MAT) using (0.2) M of 2-ME compound and 2 concentration of aminothiophenol substance (0.4, 0.5) M.

Using (0.5)M of aminothiophenol substance	**Using (0.4)M of aminothiophenol substance**	**Using (0.2)M of 2-ME compound**	**Type of infection**
96 (64)	64 (42.6)	96 (64)	Acute infection
54 (36)	86 (57.4)	54 (36)	Chronic infection
150	150	150	Total

Determination the type of infection among pregnant women in the present study depends on the level of Immunoglobulin M (IgM) in MAT. Pappas and associates (1986) indicated that the raising titer of IgM will indicate the presence of infection.

Welsh and associates (1980) noted that the immunoglobulin M (IgM) titer will rise after (2) weeks of infection and considered this as a good indicator for acute infection, where it started to appear within the first week of infection to reach its maximum level in the second week of infection then it starts to decline gradually and remain, at low level for a long period (Luyasu et al., 1995).

Table (1) also shows that the concentration (0.5)M of Amino-thiophenol substance has the same ability like the (0.2)M of (2-ME) compound in destroying the disulfied bonds joining the five arms of Immunoglobulin M (IgM) and diagnosing the type of infection among pregnant women in present study. The data also shows that the (0.4) M from the Aminothiophenol substance had a lower efficiency in diagnosing the type of infection in the samples of the study, where the rate of acute infection by using the (0.4) M of Aminothiophenol substance was (42.6%).

This is mainly due to inability of such concentrations to destroy the immunoglobulin M when it presents in a high level in the serum, in a titer ($\leq$32), since (32) cases from this sample give positive results in LAT in a titer between (32-64).

Statistical analysis indicates that there was a significant differences between the use of (0.4) M of Aminothiophenol substance as compared with (0.2) M of 2-ME and (0.5) M of Aminothiophenol in determining the type of infection by Z-test where Z=3.96 and Z>1.95 with significant level 0.05.

Table (2) shows that the (0.5) M of Aminothiophenol gives the same rate of acute infection among pregnant during the three trimester of pregnancy as compared with the results given by using (0.2) M of (2-ME) compound, since the rate of acute infection during the first trimester was (69.8%) by using both substances, in the second trimester (28.1%) and (2.1%) in third trimester.

The statistical analysis by Chi-square test indicates that there was no significant difference between the type of substance used in MAT and the period of pregnant where χ^2 = 0.218 degree of freedom df=2.

The rise of the rate of acute infection among pregnant women during the first trimester of pregnancy, similar to the result reported by Jenum and co-worker (1996) where the rate of acute infection among 35940 pregnant women was (10.9%) and the

higher rate of acute infection among pregnant women during the first trimester was reach (6.5%).

The using of MAT to determine the type of infection whether it's acute or chronic among pregnant women, especially during the first trimester of pregnancy is considered important for early diagnosis of the acute infection and subsequently to minimize the rate of abortion and congenital malformation of the fetuses by early regular treatment (Kasper, 1998).

Table 2: Rate of acute infection among pregnant women according to the period of pregnancy by MAT using (0.2) M of 2-ME compound and 2-concentration of Aminothiophenol substance (0.4, 0.5) M.

Type of substance using in MAT			**Period of pregnancy**
Using (0.5) M of aminothiophenol substance	**Using (0.4) M of aminothiophenol substance**	**Using (0.2) M of 2-ME compound**	
67 (69.8)	43 (67.2)	67 (69.8)	First Tri-mestr
27 (28.1)	20 (31.2)	27 (28.1)	Second Tri-mestr
2 (2.1)	1 (1.6)	2 (2.1)	Third Tri-mestr
69 (100)	64 (100)	96 (100)	المجموع

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