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Incidence of Rose Bengal Positive Agglutination Test Among Blood Donors in Sulaimani Blood Bank

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Abstract

This study was achieved to determine Brucella antibodies using Rose Bengal agglutination test in serum of healthy persons attending a blood bank in Sulaimani city in Iraqi Kurdistan region, each person's serum was tested directly without dilution and with 1:80 dilution, the overall positive agglutination results were observed in 29 out of 548 cases (5.29%). Twenty seven of them gave negative reaction with 1:80 dilution, we recommend that if only Rose Bengal test is used to confirm diagnosis of brucellosis, titration of patients serum is useful for better evaluation.

Keywords: - Brucellosis, Agglutination, Rose Bengal test, blood donors

Introduction

Brucellosis is a zoonotic bacterial infection caused by gram negative bacilli, in human four species , *Brucella abortus* , *B. melitensis* , *B. suis* and *B. canis* are responsible for the disease which is acquired by direct contact with secretions and excretions of infected animals and by ingesting raw milk or milk products containing viable *Brucella* organisms or infected meat. It is rarely transmitted from person to person, the disease is prevalent in rural areas, it is an occupational disease of meatpackers, veterinarians, farmers, and livestock producers [1].

Brucellosis has a worldwide distribution and high prevalence in Mediterranean countries including Iraq [2,3]. The commonest species isolated from Iraqi patients in Ninevah province have been reported as *Brucella abortus* and *B. melitensis* [4]. Infection results in development of detectable antibody response which can be detected by several serological tests [5,6].

This study is a prospective screening study to determine the presence of *Brucella* antibodies in healthy blood donors by using slide agglutination test (Rose Bengal test).

Materials And Methods

The study was conducted in a period between June to September 2000 on apparently healthy blood donors attending a blood bank in Sulaimani city, Iraqi Kurdistan region.

Questionnaire forum was filled with information from those who accepted to participate in the study, five milliliters of venous blood was aspirated into a disposable plain plastic tube, samples were allowed to clot and then were centrifuged at 5000 rpm for 5 minutes , the serum was aspirated into a plastic

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tube , haemolysed samples were discarded from the study.

A 1:80 dilution was made from the serum sample using physiological saline (0.89 % NaCl).

On a clean white plastic surface a drop from the Rose Bengal antigen(available from LINER Chemicals ,MIB Rose Bengal antigen) was mixed with an equal volume from undiluted and 1:80 diluted serum sample, after striking with a disposable plastic stirrer with slow rotatory to observe any agglutination within 4 minutes ,in each batch of samples a positive and negative control test was done.

Visible agglutination within four minutes after mixing the serum with the Rose Bengal reagent was regarded a positive reaction [7,8].

Results

The number of participants were 548 (522 male and 26 female) with male age ranged from 17 to 75 years, averaged 33.02 years while the female age ranged 18-65 years averaged 37.15 years.

The occupation of the male participants was different, among them 5 butchers and 5 farmers while 20 of 26 females were housewives and the others were employee in official works.

Of the participant, 441 (80%) were residents in city center while the other 147(20%) were resident from rural and agriculture areas. Those who gave history of brucellosis were 24 (4.3%), 20males and 4 females , while those who gave a positive family history were 44 (8.2%),40males and 4 females. Animal contacts were positive in 25 (4.5%), 23 males and 3 females. History of consumption of fresh dairy products were detected in 203, while 66 gave negative history and in 279 cases the history was not conclusive.

The number of cases who gave a positive reaction with the undiluted serum sample was 29 (5.29%), 26 males and 3 females, among whom only 2 gave positive reactions with a diluted serum (1:80).

Table 1 shows the relation of the positive and negative agglutination with the data obtained from the blood donors. Table 2 shows the result in relation of serum dilution 1:80.

Table(1) Relation of the positive and negative Rose Bengal agglutination test with The data obtained from blood donors

Criteria		No of Cases		Total Cases	% Of Positive agglutination
		Positive Agglutination	Negative Agglutination		
Gender	Male	26	496	522	4.9
	Female	3	23	26	11.5
Occupation	Related	1	8	9	11.1
	Non related	28	511	539	5.1
Residence	City center	22	389	411	5.3
	Rural	7	130	137	5.1
History	Positive	3	21	24	12.5
	Negative	26	498	524	4.9
Family history	Positive	4	40	44	9
	Negative	25	479	504	4.9

Discussion

Rose Bengal test using *Brucella abortus* bacterial suspension coloured with Rose Bengal at pH of 3.6 Sodium azide can react with both IgM and IgG antibodies produced against *Brucella abortus* , *Brucella melitensis* and other species of *Brucella* [7].The test is valuable for screening for Brucella antibodies [9]. The test is highly sensitive, figures of sensitivity between 95.79% to 100 % were reported [10]. The test must be used as a screening for brucellosis rather than the only test to diagnose *Brucella* infection if laboratory utilities were available.

Rose Bengal test is used traditionally in our locality to support or confirm diagnosis of Brucellosis, the test is done usually without diluting serum and the titer is adjusted incorrectly on visual bases, reaction strength and time needed for agglutination.

In this study the overall positive agglutination result were observed in 29 out of 548 healthy participants (5.29%) this is an expected figure and it is near the values recorded by Dabdoob (6%) among healthy control group carried by Rose Bengal test in Mousl ,Iraq [8] and by Laroche (6.6 %) in a study in Burindi using the same test [12], this is due to the similarity of these communities regarding the animal raising and the prevalence of the diseases. The higher female positive rate than male (11.5 to 4.9) is not significant due to the small number of female cases in the study.

As the serum samples were from healthy blood donor adults, the rate of positive reaction among those who gave a history of brucellosis within past 2-3 years were 12.5.% while the rate for those whose gave a negative history were 4.9% this was statistically non significant , similar result were obtained regarding family history of brucellosis .

No significant relation of occupation were observe among occupation related group, no relation with diary ingestion were obtained neither with animal exposure as the history for these relation were non conclusive .

The use of Rose Bengal test as the only test for diagnosing the brucellosis must not be practiced , and because 5.29% of cases gave a positive reaction, these may be considered as brucellosis when this test is used without titration and associated with clinical findings but all our cases were healthy normal person without any complain so it is better that if the agglutination test was positive then confirmation must be done with other tests or by titration using Rose Bengal reagent[8,12].

Table (2) the result of Rose Bengal agglutination test with undiluted and 1:80 diluted serum

Undiluted serum*	1:80 diluted serum	No of Cases
+	+	2
+	-	27
-	-	519
-	+	0

* +

*Positive, - Negative agglutination test

In the study, agglutination test were performed with undiluted serum and with 1:80 serum dilution , the value of this dilution was clear as only 2 samples among 29 positive agglutination samples still give a positive results with the 1:80 dilution. While no test was positive with 1:80 dilution while its result was negative with undiluted serum, the value of titration of serum in Rose Bengal test

is useful from the fact that using Rose Bengal test as the whole test for brucellosis diagnosis is scientifically accurate with titration rather than direct sample as the main titer done by Rose Bengal test reported among healthy control group in Dabdoob study were 1:20 although they were healthy person without the disease while the titer in true infection exceeds 1:80 to reach even four fold this figure [8] our figure is near this value as 27 out of 29 cases gave a titer below 1:80 dilution, also titration is useful specially in a case suspected to have brucellosis to exclude prozone phenomena[8].

Conclusion

Rose Bengal test is better to be performed routinely with 2 serum samples , one direct and another using 1:80 dilution as these will decrease the chance for low titer reactions and also it make a better evaluation in case of prozone in some cases who give a negative direct agglutination results .

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پشکنینی "Rose Bengal " لە خوێن بەخشدا لە بانکی خوێنی شاری سلێمانی

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ئەم لێکۆلینەوەیە بۆ دەستنیشانکردنی بوونی دژە تەنەکان دژی میکرۆبی بروسیلا (*Brucella*) لە سیرەمی خوێنی ئەو کەسە نازۆیان کە بۆ خوێن بەخشین هاتوون لە بانکی خوێنی شاری سلێمانی لە هەریمی کوردستانی عێراق ئەنجامدراوە

پشکنینی (فحصی) رۆزبینگال (Rose Bengal)بۆ ئەم مەبەستە بەکار هێنراوە بە وەرگرتنی دوو نموونە لە هەمان سیرەم . نموونەیەکیان بە خەستی و ئەویتریان بە رووکراوەیی (١ : ٨٠) . دژە تەنی دژی میکرۆبەکە لە (٢٩) کەس لە کۆی (٥٤٨) کەس واتە بە ڕێژەی (٥,٢٩ ٪) دۆزایەوە . بەڵام بەبەکارهێنانی سیرەمی رووکراوە لە هەمان (٢٩) کەسەدا دەرکەوت کە (٢٧) کەسیان دژەتەنی دژی میکرۆبەکەیان تیانەبینرا .

بۆیە لە دەست نیشان کردن (Diagnosis) ی نەخۆشی (تای مالتا) بۆ نەوەی دوو چاری هەڵە نەبیت ناموژگاری دەکریت کە سیرەمەکە رووبکرێتەوە .

أيجابة أختبار " Rose Bengal" بين المتبرعين بالدم في مصرف ألدم في مدينة ألسليما نية شيركو علی عمر

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الخلاصة

أجريت هذه الدراسة لتعيين وجود أ ضداد بكتريا "*Brucella*" في مصل أشخاص أصحاء حضروا للتبرع بالدم في أحد بنوك ألدم في مدينة ألسليما نية ،أقليم كردستا ن العراق .

أستعمل فحص " Rose Bengal" لتعيين وجود ألا ضداد و جرى أختبار كل عينة مصل بشكلها غير ألمخفف و ألمخفف بنسبة "١:٨٠" اذ وجد بان ألا ضداد أعطت نتيجة ايجابية عند ٢٩ شخص من مجموع ٥٤٨ شخص (٥,٢٩٪) عند أستعمال نموذج غير مخفف و تضمنت ٢٧ نتيجة سلبية عند أستعمال نموذج مخفف ١:٨٠ .

أستنتج من هذه ألدراسة بان الا عتماد على فحص" Rose Bengal" لتشخيص حمى مالطا يجرى بتخفيف المصل لتقيم ألنتا نج بشكل أفضل .

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