Role of samples selection in diagnosis of Brucellosis in patients with localized joint pain in Najaf province by Q PCR

دور انتخاب العينة في تشخيص مرض حمى مالطا (بروسيلا) في المرضى المصاحبين لألام المفاصل الموضعية في محافظة النجف بتقنية تفاعل تسلسل البلمرة السريع

Dr. Sabah N. AL-Fatlawi, MBChB,FIBM-Path./Immunology/Nephrology and Kidney transplant center/Al-Najaf health Directorate/MOH.
E-mail :dr_sabah_nima@yahoo.com

Abstract

Background: *Brucella* species are Gram negative, coccobacilli bacteria, facultative, intracellular organism, environmental persistence for temperature, pH, humidity, Frozen and aborted materials, they are multiple species of brucella some of them are highly weaponizable like B. sues.Brucellosis can affect any organ or system and 20-60% of cases are presented with osteoarticularcomplications(Arthritis, spondylitis,& osteomyelitis). Polymerase chain reaction (PCR) is commonly used to diagnose infectious diseases. The use of PCR for pathogens detection has high sensitivity, and high specificity, in diagnosis. PCR is the most useful assays for the diagnosis of human brucellosis.

Objective: The aim of this review was to clarify existence of pathogen of security concern like brucella spp. in biohazard region, in addition to select the most appropriate samples in diagnosis by QPCR and their relation to chronic ache.

Methodology: This study was conducted in the period between Jan. 2015 and June. 2015 in Al-Najaf city-Iraq/Al-Najaf medical private lab.this data were analysed by Chi square, Study group include 49 patients with chronic localized ache from those patients we toke 63 samples include blood, and/or tissue biopsy from site of pain, and/or CSF, blood samples were collected as 5 ml →2 ml in EDTA tube& 3 ml in serum tube, and CSF samples were collected in plane tube, while tissue biopsy submitted for DNA extraction as fresh samples immediately then these samples submitted for QPCR for diagnosis.

Results: the percentage of brucellosis in Najaf city's patients with localized joint pain was about one quarter, both tissue and blood sample can be regarded as useful diagnostic tool for diagnosis of human brucellosis by RT-PCR.

Conclusion: Brucella is regarded as endemic in Iraq and both blood and tissue samples are best selective samples in diagnosis of brucella.

Recommendation: Brucella should be considered as a differential diagnosis in cases of localized ache.

Keywords: Human brucellosis; Brucella spp; QPCR

الخلاصة:

خلفية البحث: انواع البروسيلا هي بكتريا سالبة لصبغة كرام ،بكتريا كروية-عصوية، اختيارية ،تعيش داخل الخلية ،موجودة مقاومة للحرارة و عامل الحموضة و الرطوبة، المواد التجميد اوخلال مواد الاسقاط، يوجد العديد من انواع البروسيلا جزء منها يستخدم بالتسلح بكثرة مثل بروسيلا سويس. مرض البروسيلا يمكن ان يؤثر على أي عضو او أي جهاز و حوالي 20-60 % من الحالات تظهر كمضاعفات العظام و المفاصل(الالتهاب الرئوي، التهاب العمود الفقري و التهاب العضلات و العظام).تفاعل البلمرة المتسلسل(بي سي ار) عموما يستخدم لتشخيص الامراض المعدية. إن استعمال بي سي ار لكشف الامراض له حساسية عالية و تحديد عالي الخصوصية للتشخيص. يعد البي سي ار من اكثر الفحوصات المفيدة لتشخيص بروسيلا البشر.

الهدف: الغرض من هذه المراجعة هو لتوضيح التخوف من وجود جراثيم مرضية خطرة مثل اصناف البروسيلا في المناطق الحيوية ، بالإضافة الى انتقاء العينات الاكثر ملائمة للتشخيص بتقنية تفاعل البلمرة المتسلسل السريع و علاقته بالوجع المزمن.

المنهجية: هذه الدراسة اجريت بالفترة الحصورة بين كانون الثاني لغاية حزيران 2015 في مدينة النجف في العراق في مختبر النجف الطبي الاهلي و تم تحليل النتائج بأستخدام كاي سكوير، تتضمن المجموعة الدراسية 46 مريض مصابين بالوجع الموضعي المزمن ، من هولاء المرضى تم اخذ 63 عينة تتضمن عينات من الدم و العينات النسيجية و عينات السائل الشوكي، تم سحب 5 ملي ليتر من الدم ← 2ملي ليتر من الدم في انبوبة مانع التخثر و 3 ملي ليتر في انبوبة مصل الدم و تجمع عينات السائل الشوكي في انبوبة اختبار عادية بينما يتم استخلاص مادة ا ل دي ان أي من العينات النسيجية الجديدة فورا و يتم اجراء فحص تفاعل تسلسل البلمرة السريع لغرض التشخيص.
النتائج: كانت نسبة مرض حمى مالطا (بروسيلا) في المرضى المصاحبين لألام المفاصل الموضوعية في مدينة النجف حوالي الربع. كلتا العينات النسيجة و عينات الدم تعتبر الاداة التشخيصية المفيدة لتشخيص البروسيلا عن طريق تفاعل تسلسل البلمرة الحقيقي.
الاستنتاج: تعتبر البروسيلا مرض مستوطن بالعراق و كلتا عينات النسيج و الدم افضل العينات الانتقائية بتشخيص المرض.
التوصيات: يجب ان تؤخذ البروسيلا بنظر الاعتبار كتشخيص تفاضلي للمرضى المصاحبين لألام المفاصل الموضوعية.

INTRODUCTION

Brucella is a genus of Gram-negative bacteria[(1)], they are small (0.5 to 0.7 by 0.6 to 1.5 µm), facultatively intracellar coccobacilli[(2)], which lack capsules, flagellae, endospores or native plasmids. *Brucella* is the cause of human brucellosis which is emerging as a serious animal and public health issue in many parts of the world[(3)]. Transmission of brucellosis through conjunctiva or broken skin contacting infected tissues,Blood, urine, vaginal discharges, aborted fetuses, placentas,or ingestion of raw milk & unpasteurized dairy products, also laboratory workers by inhalation, and rarely through undercooked meat,[(4)(5)].In Iraq there is a public Famous cheese which is homemade unpasteurized namedarabcheese is suspected to be the source of infection.

Brucellosis can affect any organ or organ system, and 90% of patients have undulant fever, headache, weakness, arthralgia, depression, weight loss, fatigue, and liver dysfunction. About 20-60% of cases have osteoarticular complications - arthritis, spondylitis, or osteomyelitis, while 2-20% of cases can have genitourinary involvement and sometime the patients are presented with neurological symptoms include depression and mental fatigue, evencardiovascular system can be involvedlike endocarditis.Chronic brucellosis is hard to diagnosis whilelocalized infection can occur frequently, and some cases are asymptomatic[(6)].*Brucella* species have been found primarily in mammals:[(7)]

Species	**Host**
B. melitensis	Goats and sheep
B. abortus	Cattle
B. canis	Dogs
B. suis	Pigs
B. ovis	Sheep
B. neotomae	desert woodrat(*Neotomalepida*)
B. pinnipedialis	Seal
B. ceti	dolphin, porpoise, whale
B. microti	common vole (*Microtus arvalis*)
B. inopinata	Unknown
Brucella sp. NVSL 07-0026	Baboon

B. melitensis ; Accounts for most human cases in the Mediterranean and Middle East, up to 78 cases/100,000 people/year, and represent 20% seroprevalence in Arabic Peninsula[(8)], while B. abortus is worldwide Notifiable disease in many countries most cases were presented as Fever of Unknown Origin and because of lack of recognition poor surveillance and reporting was common[(9)].

Diagnosis in Humans

Isolation of organism(Culture on Castaneda medium) from blood, bone marrow, or other tissues[10].Serum agglutination with a titer of > 1:160 in the presence of a compatible illness supports the diagnosis of brucellosis. Demonstration of a four-fold or greater increase or decrease in agglutinating antibodies over four to 12 weeks provides even stronger evidence for the diagnosis.ELISA is probably the second-most common serologic method. Immunofluorescence test(indirect) is widely used in diagnosis of brucellosis. As for other fastidious pathogens, molecular methodology offers an alternative way of diagnosing brucellosis, and define the optimal clinical specimen of human origin for this test. PCR, characterized by high sensitivity and specificity and short time can overcome the limitations of conventional methodology[11]

Q PCR(Real time PCR)

Real-time PCR is a valuable technique in determining the quantification of nucleic acids in clinical specimen of human origin. Recently, real-time PCR for the rapid detection and differentiation of *Brucella* species in clinical samples has recently been developed, targeting 16S-23S internal transcribed spacer region (ITS) and the genes coding omp25 and omp31[12], BCSP 31[13][14][15][16].

METHODOLOGY

Subjects:

This study was conducted in the period between Jan. 2015 and June. 2015 in Al-Najaf city-Iraq/Al-Najaf medical private lab.

Study group include 49 patients with chronic localized ache (small joint, localized backache in vertebra and/or headache) from those patients we toke 63 samples include blood, and/or tissue biopsy from site of pain, and/or CSF, because not all of patients were agree to give more than one sample or submitted for surgical biopsy or CSF puncture. Blood samples were collected as 5 ml →2 ml in EDTA tube& 3 ml in serum tube, and CSF samples were collected in plane tube, while tissue biopsy submitted for DNA extraction as fresh samples immediately.

PCR Kit:

BosphoreBrucella Detection Kit v1

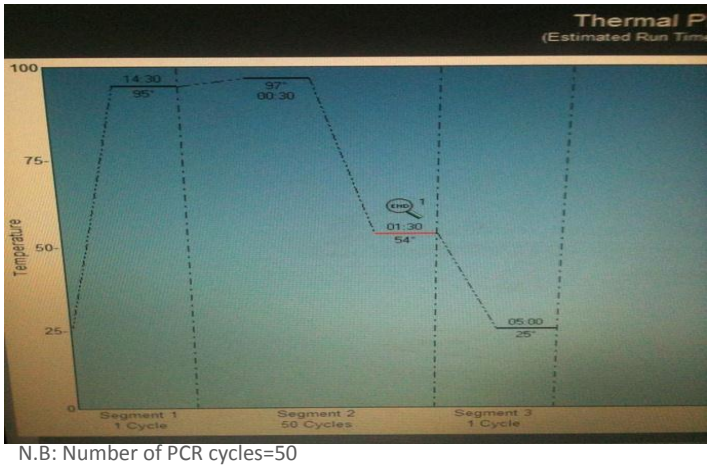
Bosphore™ Brucella Detection Kit v.1 detects Brucella DNA, encompassing all the major Brucella genotypes (B.abortus, B.melitensis, B.canis, B.suis, B.ovis and B.microti). A region within the **BCSP31 gene** of Brucella genome is amplified and fluorescence detection is accomplished using the FAM filter. The kit contains an internal control which checks PCR inhibition. The amplification data of the internal control is detected with the Cy5 filter. The internal control can be added during PCR step.

Positive control Ct=33(+/-2)

Sensitivity :7.5 x 100 copy/ml

The Agilent Mx3005P (stratagene 3005P) QPCR Systems from Agilent Technologies is the most flexible—and reliable—instruments for pathogen detection.Agilent's qPCR software, MxPro, provides users with an intuitive interface, quick experiment design, powerful data analysis and easy report generation. (Made in Germany)

Thermal profile



N.B: Number of PCR cycles=50

RESULTS

Table (1):Demographical Characteristics distributed in the patients age groups.

Variables	Samples	Age Groups/years	Frequency	Number of Positive cases	Percent of positive
Age Groups	PCR	1-20	12	1	8.33
		21-40	21	6	28.6
		41-60	11	3	27.27
		61-80	5	2	40

This table show that the majority of positive cases were higher in older age.

Table (2):Samples types in the patients Groups

Variables	Samples	Groups	Positive	Percent
QPCR	Sixty three sample From 49 patients	Blood (33 sample)	8	24.24 %
		Tissue (16 sample)	4	25 %
		CSF (14 sample)	0	0 %
		Total patient's number=49	12/49	24.48 %
		Total samples=63	12/63	19.04 %
		Number of patients with two different samples simultaneously= (14)	6/14 (3 tissue+ 3 blood)	42.85 %

***r* × *c* Contingency Table: Results(Blood,Tissue&CSF)**

The results of a contingency table X^2 statistical test

chi-square = 4.24

degrees of freedom = 2

probability = 0.120

Note:One patient has 2 tissues sample first from inflamed portion and second from the adjacent part, the former one was positive.

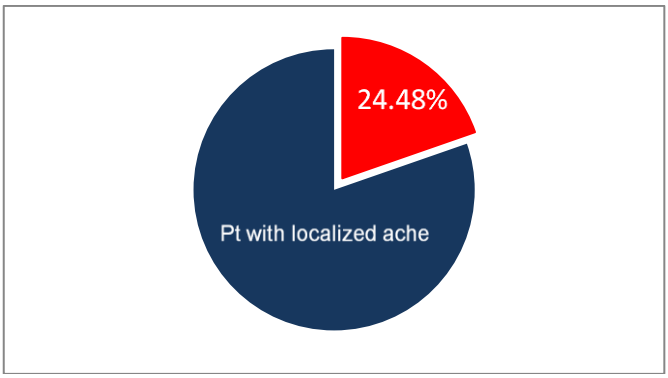


Fig.1 Percentage of Brucella in patient with localized pain

This figure is show approximately one quarter of patients with localized joint pain have brucella.

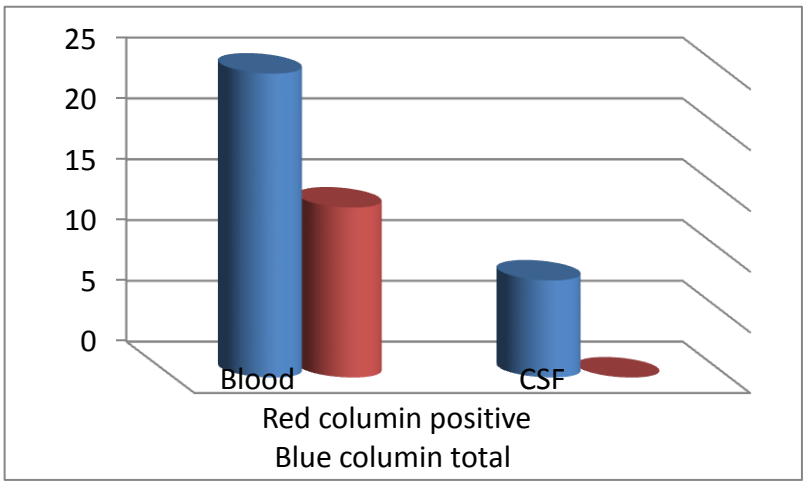
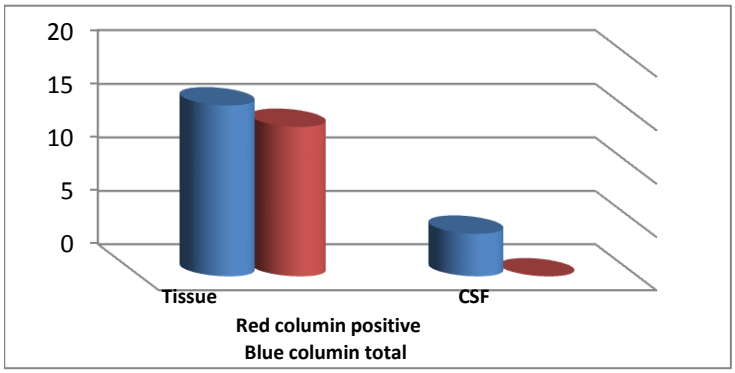


Fig.2 Percentage of Brucella in Blood and CSF

There is a significant p value between the selections of blood or tissue sample versus CSF for QPCR detection of brucellosis.



***r* × *c* Contingency Table:**
Results(Tissue & CSF)
chi-square = 4.04
degrees of freedom = 1
probability = 0.044

Fig.3 Percentage of Brucella in Tissue and CSF

DISCUSSION

majority of positive cases were higher in older age groups which is consistent with Cetinkaya*etal* [17] ,so peaks of brucella appear in elderly, due to the 'immunosenescence in elderly individuals (>65 years old). So loss function of immune system has been associated with increased susceptibility to diseases.In other hand the Brucellosis can occur at any age and also common in adolescents andyoung adults, where the majority of the population usually consume unpasteurized dairyproducts like cheese collected from rural area[18].

So this study show the percentage of brucellosis in patients with localized joint pain was 24.48 % so about one quarter of those patients in Najaf were infected, this indicate the incidence of brucella is high in Iraq ,unfortunately there is no precise statistical study about the incidence of brucellaspp in our country, but most of studies consider brucella as endemic disease in countries of the Mediterranean basin ,Arabian Peninsula, and ArabianGulf[19].

This study indicate the CSF samples have poor role in diagnosis of brucellaspp in those patients even whose had had vertebral brucellosis, and this results are inconsistent with[13], while 25 % of positive cases of brucella diagnosed through tissue samples , in other aspect about 24.24% of blood samples were positive, that is mean both tissue[20] and blood sample can be regarded as useful diagnostic tool for diagnosis of human brucellosis by PCR detection,so the RT-PCR is a confirmatory test for accurate diagnosis of individuals infected with brucellosis[21].

CONCLUSIONS

1. Brucella is endemic in Iraq and is pathogen of security of concern so the degree of awareness should be increased.
2. Blood and tissue biopsies are a recommended samples for diagnosis.
3. Tissue biopsy from inflamed portion increase the reality of test
4. CSF are poor selective sample in diagnosis of brucella.

RECOMMENDATIONS

Brucella should be considered as a differential diagnosis in cases of localized ache.

Increase awareness level for persistance of Brucella in biohazard regions because it is considered as pathogen of security of concern.

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