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The Relationship Between Infertility and Infection with Clarithromycin Resistant Strain of *Helicobacter Pylori* in Iraq

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Abstract

The relationship between infertility and *Helicobacter pylori* infection was investigated; samples from thirty-five infertile patients (aged 20-49 years) were collected from Kamal Al-Samaraei hospital , Baghdad, Iraq during the period from the first of February until April 2018. These patients were compared with 10 apparently fertile individuals who served as a control. The study was carried out to detect the DNA of *H.pylori* in both serum and seminal fluid of male infertile patients and for the control group by Real-Time Polymerase Chain Reaction (RT-PCR) technique. The results revealed that there was a significant difference ($P<0.01$) in the detection of DNA of *H.pylori* between patients and control groups. thereby the percentage level of *H.pylori* DNA in serum was 80% and in the seminal fluid was 0 %. As a result, we strongly suggest that the infection with *H. pylori* plays an important role in male infertility.

Keywords: male infertility; 23SRNA; *H.pylori*, Real time PCR, Clarithromycin.

العلاقه بين العقم الوسلالات البكتريا الملتوية البوابية المقاومة للكلاريثرومايسين في العراق

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الخلاصة

لغرض تسليط الضوء على العلاقة بين العقم والاصابة بالبكتريا الملتوية البوابية ،اجريت الدراسة على **(35)** مريض يعانون من العقم من النوع الاولي، وقورنت مع(10) من الرجال الاصحاء عند نفس الاعمار واعتبرت كمجاميع سيطرة. اظهرت نتائج التحليل الجزيئي بواسطة تفاعل سلسلة البلمرة Real Time PCR (RT–PCR) ان هنالك ارتفاعا معنويا عاليا ($P<0.01$) في نسبة انتشار الحامض النووي المنقوص الاوكسجين DNA للبكتريا الملتوية البوابية والتي بلغت 80 % في مصل المرضى الرجال العقيمين ،ايضا لوحظ ارتفاعا معنويا ($P<0.01$) في نسبة انتشار الحامض النووي المنقوص الاوكسجين DNA للبكتريا الملتوية البوابية حيث بلغت نسبت انتشاره 0% في السائل المنوي للمرضى الرجال العقيمين مقارنة مع مجاميع السيطرة. اوضحت نتائج الدراسة الحالية اهمية ودور البكتريا الملتوية البوابية في حدوث العقم.

Introduction

Gastric cancer that can be caused by *Helicobacter pylori* infection has a high distribution around the world, especially in permanent infections where it represents one of its major complications [1].

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Some researchers detected an association between bacterial infection and reproductive disorder [2]. Idiopathic infertility, which is noticed in many of infertile males, suggests the requirement for sensitive diagnostic methods [3]. Some reports concluded that dietary components may convert into carcinogens as a result of decreasing the level of gastric secretions of acids, particularly in chronic bacterial infections [4]. The presence of bacteria is usually related to successful attachment to target cells. According to area or variation, this bacterium is classified into the Western and East Asian strains [5]. Mitogen-activated protein (MAP) kinases, via epidermal growth factor receptor (EGFR) activation, can occur in response to its manipulation because of the production of several cytokines such as IL-1 and IL-6 [6].

β-defensin 3 (hBD3), one of human defense factors against bacterial infection, can be induced especially in cytotoxin-associated gene pathogenicity island. *cag*PAI bacterial strains (CagA) represent one of the most important virulence factors in *H.pylori*) [7]. The nucleotide-binding oligomerization domain-1 (NOD1) was demonstrated to play a major role in the activation of the cytokines[3]. In addition, Type four secretion systems (T4SS) may include other types of Cage like (CagL) which can be associated with α5β1 of the target cell and this lead to activation of IL-8[8]. So,β1-integrin/ FAK-independent and TGFα/EGFR-dependent manner are usually affected by this inducing [9].

The characteristics of sperms are usually disordered in bacterial infections [10]. Several reports concluded that infections with stains that express a virulence factor (CagA) elicit cytokines that lead to excessive inflammatory responses [11]. The aim of the present study is to determine the effects of *H.pylori* infection on the reproductive system.

MATERIALS AND METHODS

Patient's samples

Thirty-five infertile male individuals with a range of age of 20-49 years attended to Kamal Al-Samaraei hospital from the first of February until September 2018 .Ten samples of fertile Iraqi men of the same age range were studied as a control group . All samples were marked by the number of sample, the name of patient, and day of sample collection. Blood samples (5ml) were collected and kept at room temperature until the coagulant was formed . Then the samples were centrifuged at 3000 rpm for 5 minutes. Seminal plasma (2ml) were also collected, which is a complex fluid comprised of secretions from all organs or tubules of the seminal tract (bulbourethral glands, seminal vesicles, prostate, vasa deferentia and epididymides) and from the seminiferous tubules in the testicles. Samples were collected after centrifugation for 10 minutes and then at 300 rpm and preserved under deep freezing. The total of thirty five male infertile patients were classified into three groups; group 1 included 12 patients (34.3%) with an age of less than 30 years,. The highest number of infertility patients 14 (40 %) was located within group 2 which included an age range 30-40 years. The last group included 9 patients (25.7 %) with an age range of older than 40 years.

Molecular Diagnosis

All the study groups were included for detecting DNA of *H.pylori* in serum and semen. The extracted DNA from the serum and semen samples was submitted for bacterial detection by using qualitative RT- PCR [12,13**].** The concentrations of the extracted DNA samples from the serum and semen samples were estimated using nanodrop spectrophotometer by measuring the nucleic acid content of the samples in a volume of 1 µl. DNA absorbed ultraviolet light with an absorption maximum at 260 nm wavelength. The purity of those samples was detected by noticing the absorption at 260 nm comparing with the absorption at 280 nm. The 260:280 ratio is a good indicator of protein contamination .The real time PCR program for *Helicobacter pylori* DNA detection is shown in Figure- 1. Both FAM probe and Joe probe are the most commonly used fluorescent dyes for labeling oligonucleotides with most sensitive results and an excellent signal. Cycle Threshold (C_t).

Stage	Temp.(°C)	Time	Fluorescence detection	Cycle Repeats
Hold	95	15min.	-	1
Cycling	95	10 sec.	-	45
	60	25 sec.	Fam (Green) Joe (Yellow)	
	72	10 sec.	-	

Figure 1-The real time PCR program for *Helicobacter pylori* DNA detection

Statistical analysis

Chi-square test as a Statistical Analysis System [14] was used to identify the significant differences between patients and the control group.

RESULTS AND DISCUSSION

Both serum and semen samples of men clinically diagnosed with infertility were used for DNA extraction . Then, gel electrophoresis was used to analyze the results. The results showed sharp bands of extracted DNA. DNA was extracted from serum and semen samples using DNA-Sorb-B purification kit (Sacace Biotechnologies/ Italy), (Figure-2).

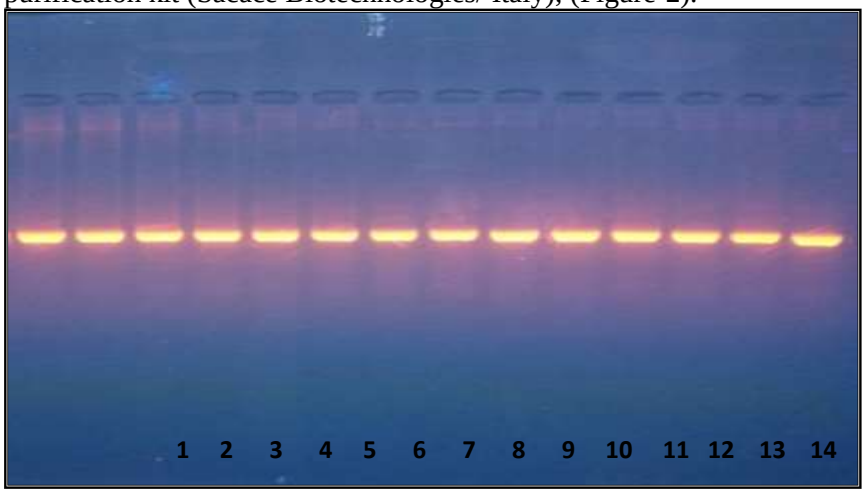

Figure 2- Gel electrophoresis of extracted DNA from serum and semen samples using 1% agarose gel at 7volt/cm for 30 hour. Lane 1-14: Extracted DNA.

A qualitative real time PCR for the extraction of DNA from the serum and semen samples was applied to diagnose the presence of bacteria Figures-(3 and 4, respectively).

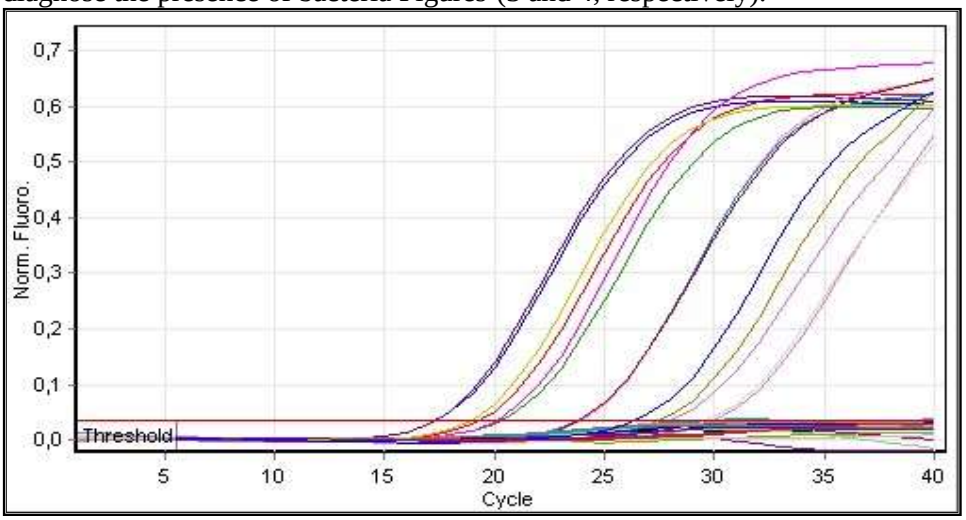

Figure 3-Schematic representation for the data of qualitative RT-PCR (Joe probe) for *H. pylori* detection.

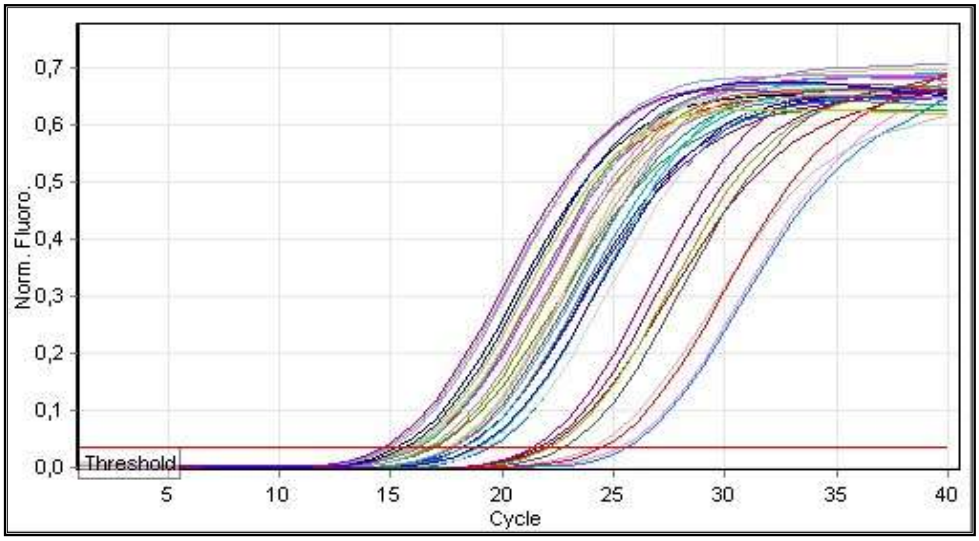

Figure 4-Schematic representation for the data of qualitative RT-PCR (FAM probe) for *H. pylori* detection.

Significant differences were recorded (P< 0.05) in the percentage of *bacterial* DNA in serum and semen (80%) and (0%), as shown in Tables- 1 and 2, respectively. The results of the current study were compatible with those of Tabriz *et al*, [15], who pointed out that DNA of *H.pylori* was not detected in the seminal fluid of infertile male individuals. An additional work is usually required to investigate the effects of bacterial infection on the activities of the reproductive system [16].

Table 1-Distribution of the serum samples according to qualitative RT-PCR of *H.pylori.*

RT-PCR	Number	Percentage (%)
Positive	**28**	**80**
Negative	**7**	**20**
Total	**35**	**100**
Chi-Square (χ^2)	**---**	**13.250 ****
P-value	**---**	**0.000`**
**** (P<0.01).**		

Table 2-Distribution of the semen samples according to qualitative RT-PCR of *H.pylori.*

RT-PCR	Number	Percentage (%)
Positive	**0**	**0**
Negative	**35**	**100**
Total	**35**	**100**
Chi-Square (χ^2)	**---**	**15.00 ****
P-value	**---**	**0.0001**
**** (P<0.01).**		

Real time-PCR is considered as one of the most sensitive techniques in the identification of bacterial DNA [17]. Quantitative real-time PCR can be used to detect rDNA with a high level of sensitivity than other techniques in individuals under treatment [18,19].

It has been found that bacterial infection play an important role by changing the polarity of target cells [20,21], inducing an immune response as a result of lymphocyte activation (22). The mutation of the positions 2,142 and 2,143 of the 23 S rRNA gene can be detected by RT-PCR, particularly the point mutations that are related to clarithromycin-resistant and –sensitive [18,23]. Several of mechanisms such as methylase production, the actions of macrolide-inactivating enzymes, and active efflux for bacterial manipulation in host cells play important roles in its pathogenicity [24]. It has been shown that the infection with CagA-positive *H. pylori* strains in women is linked to an increased potential of early abortion, while in men it is related to poor sperm quality . Although several transmission pathways have been identified for this microorganism, the definite route of transmission

is not completely understood. Recently, sexual transmission has also been proposed for this organism [25]. For example, it may colonize male urethra during sexual contacts. Because *H. pylori* infection can trigger local and systemic inflammatory responses, the presence of the bacteria in the male urethra may cause chronic inflammation, which may lead to pathogenesis. Despite of using the most sensitive and specific methods, this study could not detect any *H. pylori* DNA in the collected semen samples [26]. Several reasons can play important roles in this results, such as small sample size or very small bacterial load in the selected samples, which could not be detected by PCR, as well as the actual absence of this bacterial colonization in male urethra. We recommend in the future studies to diagnose infertility in women using PCR technique to detect 23rRNA mutation caused by the infection with clarithromycin resistant *H.pylori.*

CONCLUSION

Finally, in general, we conclude that the infection with *H.pylori* can have a direct effect on impairing reproductive system activities.

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