DETECTION OF *HELICOBACTER PYLORI* IN ATHEROMA OF ISCHAEMIC HEART DISEASES PATIENTS IN THE SOUTH OF IRAQ BY PCR TECHNIQUE

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ABSTRACT

Ischaemic heart disease (IHD) or coronary heart disease (CHD), It is the end result of the accumulation of atheromatous plaques within the walls of arteries that supply the myocardium; Recent study confirmed identification of *H. pylori* deoxyribonucleic acid (DNA) in atherosclerotic plaques of patients with severe coronary artery disease, which supports the hypothesis that *H. pylori* infection may influence the development of atherosclerosis. The study aimed to detect presence of *H. pylori* DNA in the atheroma or intima of 50 subjects (which were divided into two sub groups: persons with IHD and persons without IHD) by PCR technique, the study showed that 3% of persons with IHD and 6% of persons without IHD had positive results and 4% (from all subjects) of study, without regard to group type, had positive results.

KEYWORDS: *Pylori* deoxyribonucleic,

INTRODUCTION

Helicobacter pylori are gram-negative, unipolar, multiflagellate, microaerophilic, gently spiral or curved bacilli, measuring approximately 3.5 microns in length and 0.5 microns in width (Mobley, 1996). The organism has two to seven unipolar sheathed flagella which enhance its mobility through viscous solutions (Goodwin & Worsley, 1993). It is a slow growing organism that can be cultured on blood agar or selective media such as Skirrows media incubated at 37℃ in a 5 % oxygen atmosphere for three to seven days (Marshall &Warren , 1984). The organism can be biochemically characterized as catalase, oxidase,

and urease positive (Amieva& Omar, 2008). It has been suggested that sheep may be a natural host for *H. pylori* (Dore *et al.*, 2001). Current epidemiologic evidence indicates most infections are acquired during childhood even in Western countries (Pounder, and Ng, 1995 and Parsormet, 1995). The prevalence of *H. pylori* among adult population in the Middle East was estimated to be in the range of 70 to 90% (Novis *et al.*, 1998). It is suggested that *H. pylori* may be hyper-endemic among arab patients with dyspepsia (Rashed *et al.,* 1992). Studies have revealed that the pathogen is present in a large proportion of people. It occurs in the stomach of 25% of healthy middle-aged adults and in more than 60% of adults over 60 years of age (Kathreen And Arthur, 2005). Researchers throughout the world focused on diseases related to this bug not only in gastroenterology, but also in other disciplines such as cardiology, neurology, and dermatology (Wadstrom *et al.*, 1996; Logan, 1996 and Rain *et al.*, 2001).

The heart is an aerobic organ that is dependent for its oxygen supply entirely on coronary perfusion (Munther &Homoud, 2008). Myocardial ischemia is an imbalance between the supply (perfusion) and demand of the heart for oxygenated blood. In most cases there is a long period (up to decades) of silent, slow progression of coronary lesions before symptoms appear. Thus, the syndromes of IHD are only the late manifestations of coronary atherosclerosis that may have started during childhood or adolescence (Robbins and Cotran, 2010). Ischemic heart disease is caused by narrowing of one or more of the three major coronary artery branches. These are functional end-arteries, and sudden occlusion of any one leads to infarction in the area of supply (Parakrama and Clive, 1998). Two variants of clinical presentation of ischaemic heart diseases: angina pectoris (which is subdivided into: typical or stable angina, prinzmetal or variant angina and unstable angina or called crescendo angina) (Robbins, 2007). and myocardial infarction (MI) (Boon *et al*., 2007).

MATERIALS AND METHODS

The subjects of this study are (50) persons, whose ages ranged between 45 to 70 years, submitted to catheterization or percutaneous intervention (PCI) in AL- Nasseriya center for cardiology from September 2012 to April 2013. Two divisions of subjects: with ischemia and without ischemia, according to PCI and catheterization.

Aduplicate for each subject by using previously autoclaved slides lense papers for PCR by friction with catheter or wire after the end of diagnostic catheterization or percutaneous

intervention, then the lense papers that were collected aseptically, were put in sterile eppendorf tube in deep freeze until use.

DNA extraction was done on above lense paperes according to Geneaid company kit instructions (manufactured in USA) to extract the DNA of supposed *H.pylori* that may be present in coronary arteries of subjects. The detection of universal 16srRNA gene of *H.pylori* by oligonucleotide primer and amplifying these gene by PCR technique apparatus (Thermal cycler) (from Labnet company in USA), the primer sequence (5-3) direction for both forward and reverse primers respectively: 5-GCTATGACGGGTATCC-3, 5-GATTTTACCCCTACACCA-3 with size product 400 bp (Fukuda *et al.,*2002) . The reaction programs were performed according to Fukuda *et al.,* (2002) for 16srRNA gene for *Helicobacter pylori* in PCR thermal cycler apparatus as shown in tables (1).

Table 1: a program for universal 16srRNA gene of *H.pylori*

Steps	Temperature	Time	No. of cycles
Initial denaturationn	95 ℃	5min	1
Denaturation	94 ℃	1min min	
Annealing	55 ℃	30 sec	40
Extension	72 ℃	1min	
Final extension	72 ℃	5min	1

PCR amplification products were determined by agarose gel electrophoresis according to Sambrook and Russell, (2001). Statistical package for social science version 19 (SPSS 19) was used for data input and analysis, Chi square test was used to test the significance of association between two discrete independent variables distribution. P-value used was asymptotic and was two sided. Findings of P-value at levels less or equal to 0.05 was considered significant.

RESULTS

In our study we determined presence of specific 16SrRNA gene of *H.pylori* from atheroma in persons with IHD and intima in persons without IHD as negative or positive results as shown in table (2) below.

Table 2: results of PCR test in study groups for 16SrRNA gene of *H,pylori*

Study group	*H.pylori* specific 16SrRNA gene		Total	P-value
	Positive +ve	Negative -ve		
With IHD	1	33	34	
	3%	97%	100%	0.578

Without IHD	1	15	16	
	6%	94%	100%	
Total	2	48	50	
	4%	96%	100%	

In table above we had 3% of persons with IHD and 6% of persons without IHD had positive results and 4% from all persons of study, without regard to group type , had positive results with little difference not reach us to significancy, P-value= 0.578.

Figure (1) show the distinction of 16srRNA gene of *H.pylori* in the atheroma of persons with IHD and the intima of persons without IHD by agarose gel electrophoresis.
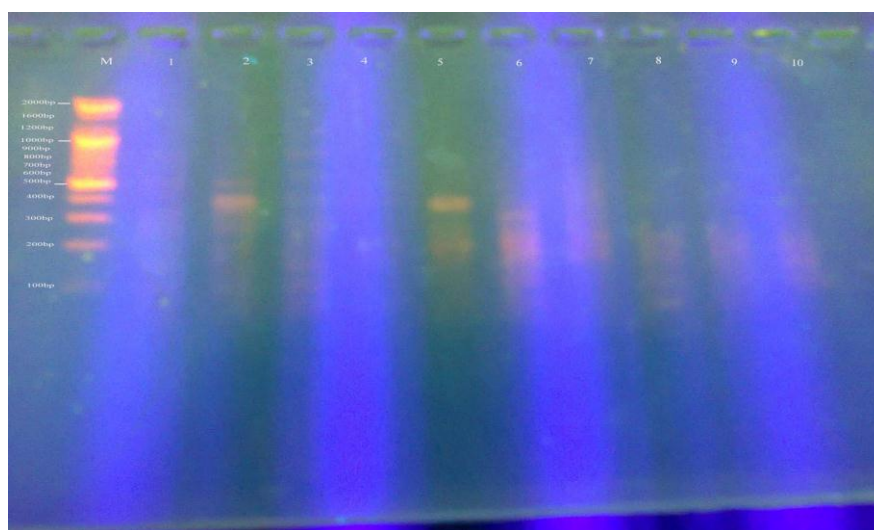

Figure 1: Agarose 2% gel electrophoresis pattern shows PCR amplified products of *H.pylori* 16srRNA gene: lane M 100-2000 bp DNA ladder; lanes 2& 5 positive samples 400 bp; other lanes negative samples.

DISCUSSION

During recent years, molecular techniques have been widely used in order to detect *H. pylori* and other *Helicobacter* species (Chisholm and Owen, 2003). Polymerase chain reaction (PCR) was also used in order to determine the presence of *H. pylori* in tissues other than gastric biopsies (Streutker *et al*., 2004), the direct proof of bacteria in arteries of affected organs seems to be more convincing, as the previous failure to demonstrate the presence of *H.pylori* in atherosclerotic plaque materials (Blasi *et al.,* 1996). Because of the highly sensitive and specific molecular technique (PCR), the results must be taken in mind and modify the design of later studies to reach the fact of presence of DNA segment of *H.pylori* in atheroma or intima of person with or without IHD respectively , because we know

that the development of atherosclerosis started early in childhood until reached to a certain level of plaque to be detectable by catheterization which may be substituted in later studies by more sensitive methods to detect atherosclerotic plaques such as angioscopy and intravascular ultrasound , and thus the persons (without IHD in the study) who exhibited presence of _H.pylori_ DNA in their coronary arteries , might be about to get clinically evident aschaemia. This study agreed with (Adiloglu _et al._, 2005) and (Ranjit _et al._, 1999).

Finally, all what we noticed in the introduction belongs to relation of _H.pylori_ and IHD is controversial and we said according to our study that it is controversial to yet, further studies needed with different designs and ideas to be done to reach the exact fact.

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