

DETECTION OF HELICOBACTER PYLORI IN MIDDLE EAR CLEFT AND ITS POSSIBLE ROLE IN CHRONIC SUPPURATIVE OTITIS MEDIAMohamed Abdel Moneim*¹, Hatem El habashy¹, Osama Refaat¹, Ahmed Abdel tawab² and Osama Mostafa³¹Dept. of ENT, Faculty of medicine Al Azhar university.²Dept. of Microbiology, Faculty of medicine Al Azhar university.³Dept. of General Pathology, Faculty of medicine Al Azhar university.

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ABSTRACT

Aim of the work: considering the close relationship between GERD and tubotympanic disorders, the presence of helicobacter pylori in middle ear cleft and its possible role in pathogenesis of CSOM has to be investigated. **Patients and Methods:** this prospective study was carried out at Al-Azhar University hospitals during the period from June 2014 to June 2016. forty patients were enrolled in this study, 25 (62.5%) were males and 15 (37.5%) were females. their age ranged from 18-58 years with a mean 36 ± 2 years. the control group was consisted of 20 healthy persons with a mean age 22.6 years (range 19 - 29 years). written consent was taken from all patients and control subjects. CLO test was performed to all patients. 14C UBT was performed. Immunohistochemical staining to all specimens were performed using H. pylori antibody. **Results:** the CLO test was positive in 24 (60%) patients, and 14CUBT was positive in 26 (65%) patients. There was a significant positive correlation between CLO test results and 14C UBT results in the patients group ($p < 0.002$). The 14C UBT result of the patients and control were significantly different ($p < 0.001$). There were only 3 cases (7.5%) positive H. pylori by immunohistochemical study. **Conclusion:** The results of the present study does not support a role for H. pylori infection in pathogenesis of CSOM. Further studies are needed to confirm the presence of the organism in middle ear cleft and its role in pathogenesis of COM.

KEYWORDS: GERD and tubotympanic, Immunohistochemical staining.**INTRODUCTION**

Chronic suppurative otitis media (CSOM), is a middle ear mucosal disease characterized by intermittent or persistent, chronic mucopurulent discharge through a perforated tympanic membrane and can be associated with cholesteatoma. Cholesteatomais a benign and destructive lesion of the middle ear, characterized by the progressive accumulation of hyperproliferative epithelium with keratin (Huisman et. al. 2003). Bone lysis and recurrence are relevant features in the pathophysiology of this disease, making it dangerous, debilitating and difficult to treat.

Helicobacter pylori (H. pylori) is a microaerophilic, gram negative, spiral microorganism that was discovered in 1984 by Marshal and warrens from ulcers of the stomach. Since that time much attention has been paid to this organism and its infection. H. Pylori is not a commensal organism as it causes gastritis characterized by infiltration of both acute and chronic inflammatory cells (Suzuki et. al. 2003) It may leads to carcinoma at the site of ulcer and mucosa – associated lymphoid tissue in long term infection (Czesnikiewicz et. al. 2004).

Although, there is no accurate incidence for the infection with H Pylori, it was assumed that the bacteria can be found in approximately 50% of the world population (Jiang et. al. 2004).

Colonization of H. Pylori has been found in the oral cavity in saliva and dental plaque (Nguyen et. al. 1995), in the tonsil and adenoid acts as a reservoirs of the organism (Cirak et. al. 2003), in the ethmoid and maxillary sinuses suggesting its role in chronic sinusitis (Ozdek et. al. 2003) and also in middle ear effusion in children with otitis media with effusion (karlidag et. al. 2005). Possible modes of transmission are through the oral_ oral, fecal_ oral and gastrointestinal _oral routes (Feldman et. al. 1997).

It is believed that gastro-esophageal reflux disease (GERD) is a predisposing factor in most upper respiratory problems including pharyngoesophagitis, croup, rhinosinusitis, laryngitis and otitis media with effusion. The relationship between chronic tubotympanic disorders and GERD has been determined but the underlying mechanism is still unknown (Rozmanic et. al.

2002). The impact of gastric *H. Pylori* infection on the pathogenesis of GERD is controversial. *H. Pylori* can survive for a certain period in the gastric juice in the esophagus and systemic immune response to gastric *H. Pylori* infection might play a causative role in the upper respiratory disease (Yemison *et al.* 2012). It has been found that *H. Pylori* impairs T cell _ mediated immunity via systemic mechanisms (Salama *et al.* 2013).

The relationship between *H. Pylori* and otitis media with effusion (OME) was previously described but the presence of *H. Pylori* in middle ear tissue and its role in pathogenesis of CSOM is presently under investigations. Considering the close relationship between GERD and tubotympanic disorders, presence of *H. Pylori* in middle ear cleft and its possible role in pathogenesis of CSOM has to be investigated.

PATIENTS AND METHODS

This prospective study was carried out at Al Azhar university hospitals during the period from Jun 2014 to Jun 2016. Forty patients were enrolled in this study; 25 (62.5%) were males and 15 (37.5 %) were females. Their age ranged from 18 to 58 years with a mean 36 ± 2 years. The control group was consisted of 20 healthy persons with a mean age 22.6 years (range 19 - 29 years). There were 8 females and 12 males. written consent was taken from all patients and controls.

All patients were diagnosed as having CSOM with or without cholesteatoma by history, clinical, radiological (CT Scan) and audiological examination. All patients were asked for symptoms of GERD (Heart burn, Regurgitation, Epigastric pain) and history of peptic ulcer. Patients use H2 receptor blockers, antacids or antibiotics within the previous 4 weeks before surgery were excluded. Patients were classified into two groups:

Group I: patients of CSOM with cholesteatoma (20 patients). 14 patients (70%) were males and 6 patients (30%) were females. Tympanomastoidectomy operations according to the pathology of ear disease were done for this group.

Group II: patients of CSOM without cholesteatoma (20 patients). 11 patients (55%) were males and 9 patients (45%) were females. Tympanoplasty operation were done for this group.

Tissue samples were obtained from the middle ear mucosa and cholesteatoma matrix during surgery, great care was taken to avoid contamination and collected samples were placed into the paraffin blocks and transported to pathology department for histopathological examination. Another tissue samples were sent to microbiology department for bacteriological study.

Bacteriological Study

A) Materials used for transportation, isolation and identification of *H. pylori*

1. 1-Transport medium: 1ml of sterile brain heart infusion broth in small plastic screw capped bottles put in ice bags and transported directly to microbiology department.
2. Medium for isolation of *H. pylori* from tissue samples
 - a) Dent's medium and brain heart infusion.
 - b) Helicobacter pylori selective supplement (Dent's supplement vial) which has the following constituents:
 - Vancomycin 5.0 mg.
 - Trimethoprim lactate 2.5 mg.
 - Cefsolodin 2.5 mg.

The supplement was hydrated by addition of 2ml sterile distilled water and mixed gently. The vial was sufficient to supplement 500 ml of the medium.

3. Media for identification of isolated organisms
 - * Medium for rapid urease test (modified Christensen's urea test)
 - It was prepared as follows:
 - Sodium chloride 0.5 gm.
 - K₂HPO₄ 0.2 gm.
 - Distilled water 99.0 ml.
 - Urea 40% 6.0 ml.
 - Phenol red 0.4% 1.0 ml.

These ingredients were mixed together and stored in refrigerator in small plastic tubes at -4°C.

4. Reagents for identification of isolated organism:
 - a) Gram stain.
 - b) Oxidase and catalase test.
 - c) Gas generating kits for reduced oxygen atmospheres (Microaerophilic) in anaerobic jars (Oxoid, Gas generating kit campylobacter system BR 056A).



Figure 1: Microaerophilic culture of *H. pylori* on brain heart infusion.

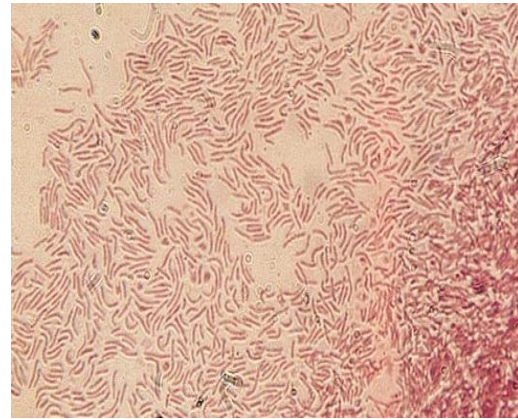


Figure 2: *Helicobacter pylori* Gram stain from culture.

CLO test (*Campylobacter-like organism test*) or rapid urease test

CLO test presents the advantage of yielding results in 1–24 hours making it a suitable method to detect *H. pylori* in epidemiological studies (Fakhrjou *et al.*, 2011). In the presence of *H. pylori* urease, urea is hydrolyzed to produce ammonia and bicarbonate, leading to a pH increase in the gastric mucosa, which is indicated by a change in the color of phenol red from yellow to pink or red (Steven, 2005).



Figure 3: *H. pylori* urease test (by inoculation from culture on solid urease test).



Figure 4: *H. pylori* urease test (by inoculation from culture on fluid urease test).



Figure 5: *H. pylori* CLO test (rapid urease test) by direct reaction from the samples.

14C Urea Breath Test (14C UBT)

The capsules of urea labeled with 1 μ Curie of [14C] (HeliCap Urea Bulk Capsules, produced for Noster System AB, Stockholm, Sweden) for 14C UBT procedure were used. The patient swallowed the urea capsule with 20mL of water. After 10 minutes, the patient was asked to inflate the test bag (Heliprobe Breath Card, Noster System AB), and then this Breath-Card was inserted to Gieger-Müller counter (Heliprobe-analyser, Noster System AB), and activity was counted for 250 seconds. The results were assessed according to grading system suggested by the producer firm (0, negative; 1, uncertain; and 2, positive).

Histopathological and Immunohistochemical study

Four micron thick sections were cut from paraffin specimen blocks of all cases and stained with haematoxylin and eosin (H and E) and histologically examined. For immunohistochemical assay, sections were taken on poly-L-lysine coated adhesive slides (Sigma, USA). Deparaffinization and rehydration were performed. For enhanced antigen retrieval in formalin-fixed, paraffin-embedded tissues, we used the microwave-oven heating method. Endogenous peroxidase of each section was inhibited with 0.3% solution hydrogen peroxide in methanol for 30 minutes. Ultra V block was applied for 5 minutes.

The primary antibody, used, was: anti *H. pylori* antibody (Polyclonal Rabbit Anti-Helicobacter Pylori, Dako; Glostrup; Denmark, 1:100 dilution, *H. pylori* infected gastric epithelium as positive control with sensitivity for *H. pylori*: 85 % and the specificity: 90.0 % and FLEX negative control rabbit as negative control),

The streptavidin-biotin horseredish kit (Dako, USA) was used for immunohistochemical staining (detection of bounded antibodies). The sections were washed with phosphate buffer solution (PBS) to prevent from drying. substrate/ chromogen (DAB) mixture was added. Counterstaining with Harris's haemtoxylin was done. Specimens were dehydrated in alcohol and cleared in xylene and mounted by DPX then slide covers were put.

Under the light microscope, sections were examined and a case was considered positive when brown reaction was developed.

RESULTS

Bacteriological results

There were no statistical significant difference between the age and sex of the patients and controls. The CLO test were positive in 24 (60%) patients. The 14C UBT was positive in 26 (65%) patients. 14C UBT was performed for 16 patients who had negative CLO test, eight of them (50%) had a positive results. There were a significant positive correlation between CLO test results and 14C UBT results in the patients group ($p < 0.002$). The 14C UBT results were positive in 8 (40%) of controls. The 14C UBT result of the patients and controls were significantly different ($p < 0.001$).

Histopathological results

Histological study of the CSOM with cholesteatoma patients revealed keratin debris and keratinized fully differentiated, squamous epithelium (matrix) similar to the epidermis of skin, resting on connective tissue. The subepithelial connective tissue (perimatrix) showed chronic inflammatory infiltrate (lymphocytes, neutrophils, macrophages, and plasma cells), cholesterol clefts, foreign body giant cell granulomas and hemosiderin pigments.

Histological study of the CSOM without cholesteatoma patients revealed thin connective tissue structures lined by attenuated keratinizing squamous epithelium. The

subepithelial tissue showed moderate to marked, mixed acute and chronic inflammatory infiltrate composed mainly of lymphocytes, plasma cells and neutrophils. Some cases showed granulation tissue composed of capillaries and fibroblasts with inflammatory background. No specific granulomas could be detected.

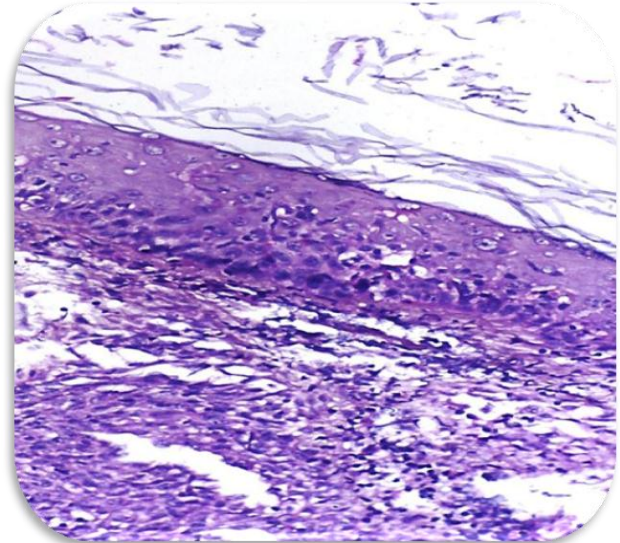


Fig 6: Histological appearance of a case of cholesteatoma composed of keratin debris and mature stratified squamous epithelium overlying inflamed stroma (HandEx200).

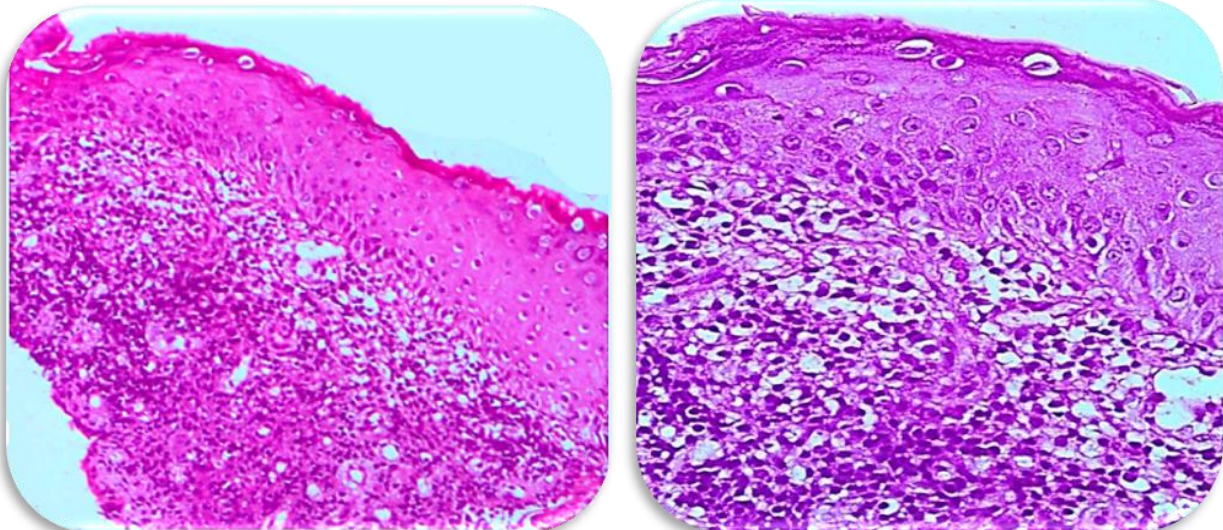


Fig 7: and Fig 8: Histological appearance of a case of CSOM without cholesteatoma: composed of connective tissue lined by keratinizing squamous epithelium and showing granulation tissue with inflammatory background. (HandE x100)and (HandE x400), respectively.

Immunohistochemical results

H. pylori were identified in only three (7.5%) of patients, one (5%) of the twenty cases of CSOM with cholesteatoma and in two (10%) of the twenty cases of

CSOM without cholesteatoma. *H. pylori* were identified as curvilinear bacilli showing strongly positive reaction for *H. pylori* antibody.

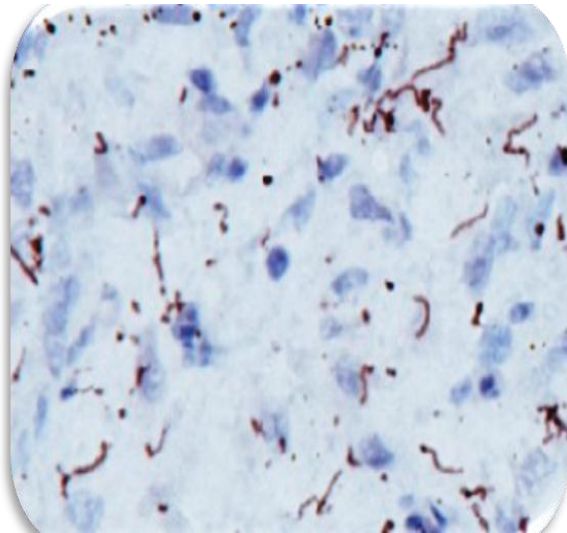


Fig. 9: A case of CSOM showing positive H. pylori immunohistochemical staining at the perimatrix (x1000).

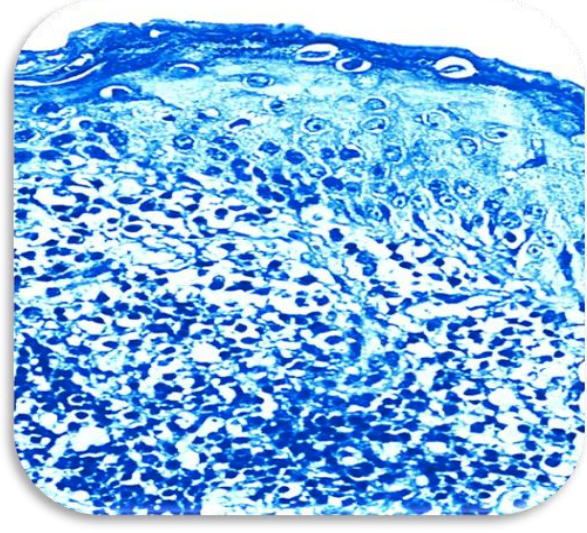


Fig. 10: A case of CSOM showing negative H. pylori immunohistochemical staining at the perimatrix (x400).

DISCUSSION

H. pylori has been argued to be the most common bacterial infection in humans (Thomas *et al.* 1992). It could be found in approximately 50 % of the world population (Jiang *et al.* 2004). Diagnostic methods are categorized into two groups as invasive and non invasive tests. The invasive tests include histological diagnosis, CLO (rapid urease) test, culture and polymerase chain reaction (PCR). Non invasive tests include 13,14C UBT and antibodies tests.

14C UBT is a very accurate test for detecting *H. pylori* infection with a sensitivity and specificity better than many other tests. The organism produces a large amount of urease, an enzyme that breaks down urea to form ammonia and soluble carbon dioxide, which is expired in the exhaled breath. Labeling of urea with isotope allows the 14C to be detected in the expired breath. The CLO test is also a very accurate test for detecting *H. pylori* with 90% sensitivity and 100% specificity rate (Loster *et al.* 2006).

Human stomach was considered to be the only reservoir of *H. pylori* until bacteria were discovered in human dental plaque, oral lesions and saliva (Kutulhan *et al.* 2005). It is believed that GERD is a predisposing factor in most upper respiratory problems and the nasopharyngeal content can enter the middle ear via Eustachian tube which is becoming popular in recent years. This could be due to the negative pressure in the middle ear cavity produced by Eustachian dysfunction or the high nasopharyngeal pressure produced at proximal end of Eustachian tube (Bluestone and Klein 2000). It was proposed that the gastric contents in the nasopharynx might cause Eustachian dysfunction and nasopharyngeal inflammation. Wittenborg and Neuhausers (1993), Demonstrated that fluids in the nasopharynx entered the nasopharyngeal orifice of

Eustachian tube in normal physiologic state. Exposure of ciliated respiratory epithelium found in Eustachian tube to a gastric juice with PH of less than 4 results in ciliostasis and this impairs mucociliary clearance, this impairment can cause colonization of *H. pylori* in the middle ear.

The relationship between *H. pylori* and OME was fully described by Karlidag *et al.*, (2005) who determined the presence of *H. pylori* in middle ear effusion of patients with OME by polymerase chain reaction (PCR). They collected 55 aspiration samples from 38 children with OME with age ranging from 2 to 12 years. Nine (16.3%) of them were positive for *h. pylori* by PCR. They concluded that *H. pylori* was detected in the middle ear effusion of some patients with OME. These results may have interesting implications for a possible role of *H. Pylori* in OME. Furthermore, Agirdir *et al.* (2006) collected middle ear effusion from 30 pediatric patients with OME, and washed middle ear of 15 age matched patients without middle ear effusions. They detected *H. pylori* in 20 (66.6 %) patients with OME, but not in washed of the middle ear patients without effusion according to the CLO test. Yilmaz *et al.* (2006) aspirated the middle ear fluid from 22 patients with OME and a small biopsy was taken from the promontorium mucosa for all patients. For the control group (20 patient) myringotomy was done and a small biopsy was taken from the promontorium mucosa. For both groups, 5mm deep tissue specimens were obtained from tonsil and adenoid tissues. For all the specimens taken from the patient they concluded that significantly increased colonization by *H. pylori* of middle ear in patients with OME indicates that bacteria reaching the middle ear through GER might be involved, culture and a nested PCR were performed to show *H. pylori*. Middle ear fluid culture was positive for *H. pylori* in 2 patients and mucosa cultures was positive in 1 patient only. In the control group middle ear mucosa cultures were always

negative. When cultures and PCR results were combined together the middle ear was positive for *H. pylori* in 10 patients in the study group and in 2 patients in the control group. This difference was statistically significant. *H. pylori* in the tonsillar and adenoid tissues by culture and PCR was also significantly more frequent in the study group compared to control group. They concluded that significantly increased colonization by *H. pylori* of middle ear in patients with OME indicates that bacteria reaching the middle ear through GER might be involved in pathogenesis of OME. *Morinaka et al., (2005)* in their study on middle ear fluid of patients with otitis media with effusion, found that twelve of 15 smears were positive for *H. Pylori* by immunohistochemistry and they concluded the presence of *H. pylori* in the middle ear fluid of patient with otitis media with effusion.

Moreover, for OME cases resistant to medical treatment it may be meaningful to evaluate the patient for GERD and *H. pylori*. On other hand, *Fancy et al. (2009)* confirmed the presence of *H. Pylori* in the nasopharynx and middle ear space, (10 of 45 Patient of the study group and 6 of 37 controls ($p = 0.44$)). But the results don't support a role for this bacterium in the pathogenesis of OME. Recently, A PCR – based study of aspiration samples collected from adult patients showed that 24 (40%) were *H. pylori* positive (*Bai et al. 2012*). These findings suggest that *H. pylori* could be responsible for the etiopathogenesis of OME. However, the relation between *H. pylori* and OME remains controversial.

The presence of *H. pylori* in middle ear tissue and its role in pathogenesis of chronic suppurative otitis media (CSOM) is presently under investigations. There are two clinical studies investigating *H. pylori* in patients with COM (*Kutulhan et al. (2005)*). Investigated a possible contribution of *H. pylori* to the etiopathogenesis of COM. Ear biopsy specimens were obtained from middle ear, mastoid antrum and tympanic orifice of Eustachian tube. *H. pylori* DNA was extracted from biopsy specimens by nested polymerase chain reaction. This study involved 38 patients (22 male and 16 female) with COM (17 with and 21 without cholesteatoma). Three (7.9%) of 38 patients were found to be positive for *H. pylori* DNA. These patients were devoid of cholesteatoma. Nine (23%) of 38 patients had classic complaints of GER. *H. pylori* was detected in gastric mucosa of eight (88%) of nine GER positive patients. Three (37.5%) of these eight patients had simultaneously a positivity for *H. pylori* in the ear. They concluded that even though it is possible to detect *H. pylori* in middle ear cleft in COM, its role in the etiopathogenesis of the issue is controversial. *Dagli et al. (2006)*, investigated the presence of *H. pylori* by CLO test in the middle ear of the patients with COM. they also investigated the relation between *H. pylori* in the stomach and in the middle ear by 14C urea breath test for the possible source of this bacterium. Tissue samples were obtained from the middle ear mucosa of 41 patients undergoing ear surgery for CSOM and placed in the CLO test Kit.

14c UBT was performed in 24 patients and 20 normal subjects. They found that CLO test results were positive in 22 patients (53.6%). Results of 14CUBT that was performed in 24 patients were positive in 19 patients (79.1%). 14C UBT was performed in 14 of 22 patients who had positive CLO test result. The 14c UBT results of these 14 patients were positive (100%). There was a significant positive correlation between CLO test results and 14C UBT in the patients group ($P=0.002$). The 14c UBT results were positive in 6 control subjects (30%). The 14c UBT results of the patients and controls were significantly different ($P=0.001$). They found that 53% of middle ear mucosa obtained from 41 patients were shown to be positive for *H. pylori*, and positivity rate of 14C UBT result was 79.1% in patient group. The relation between *H. pylori* in the stomach and in the middle ear was also investigated for possible source of this bacterium, and their results suggest that the source of this bacterium is the stomach. The current study demonstrated an association between *H. pylori* and CSOM, but it could not show a causal relationship. Further clinical studies are needed for demonstrating the pathogenesis.

The results of the present study were in consistent with the above results as the CLO test were positive in 24 (60%) patients, and positivity rate of 14C UBT result was 65% in patient group and 40% in the control group. The difference between the positivity rates of 14C UBT result of the patient and control groups were statistically significant ($p < 0.001$). Furthermore, *Dagli et al. (2006)*, suggest that the source of this bacterium is the stomach. The results of the present study agree with this concept, because, the positivity rate of 14C UBT in the patient group is considerably higher than that of the control group.

On the other hand, the immunohistochemical study results of the present study were nearly the same results of *Kutulhan et al. (2005)*, were only 3 cases (7.5%) of CSOM were positive for *H. pylori* antibody. Also *Sutton et al, (2015)* who use anti-*Helicobacter pylori* clone (SP48) (Ventana Medical Systems) for detection of *H. pylori* in cholesteatoma patients, found that of the 30 patients they examined, *H. pylori* was not identified on any of the cholesteatoma specimens. As regard these data, they suggested that there is no role of *H. pylori* in the etiology of cholesteatoma.

This means that, there is a contradiction between the results of the CLO test and 14C UBT and the immunohistochemical results. This may be due to lack of presence of the organism in tissues specimen or small tissue specimen for identification. This results does not support a role of *H. pylori* infection in pathogenesis of CSOM. Further studies were needed to confirm the presence of the organism in the tissues and its role in pathogenesis of CSOM.

Furthermore, there are two studies examined the role of *H. pylori* in experimental animal model of otitis media. Live *H. pylori* or physiological saline was added to the middle ear cavities of white rabbits, inducing otitis media. A further injection of live *H. pylori* induced accelerated inflammation in the middle ear with animals that had been injected with histamine (Aycicek *et al.* 2012). Another study found that the direct injection of protein extracted from whole cell sonicates of *H. pylori* (American type culture collection) into the middle ear of mice induce regulation of inflammatory cytokines (macrophages migration inhibition factor, macrophage inflammatory protein, interleukin -1B and tumor necrosis factor alpha). As well as sever inflammatory cells in the middle ear epithelium (Karia *et al.* 2008). These findings suggest that *H. pylori* plays a role in the development of middle ear inflammation, although the bacterium is not live.

The inflammatory response to *H. pylori* infection in the stomach has been clarified. Among the known *H. pylori* virulence factors that include cytotoxin – associated gene A product (cag A), vacuolated cytokines (vac A), outer inflammatory protein and duodenal ulcer promoting factor (Yamaoka 2010). Vac A and cag A have been investigated in an effort to understand the pathogenicity of this bacterium. Vac A is a performing to disrupt cell polarity in the gastric mucosa promotes the apoptosis of epithelial cells, and inhibits T cell proliferating cells. Cag A is an immune dominant antigen that is translocated into gastric epithelial cells through the cag type IV secretory system encoded by cag pathogenicity islands (Delahey and Rugge 2012). *H. pylori* strain that express cag A are associated with an increase of gastric cancer.

Systemic immune and inflammatory response might be responsible for extra gastric disease. CSOM develops from a chronic bacterial infection, and the mechanism of infection of the middle ear is postulated to be translocation of bacteria from the external auditory canal through a tympanic membrane perforation into the middle ear, but some authors suggest that the pathogenic organisms may enter through reflux of the Eustachian tube (Meyerhoff *et al.* 1978). Dagli *et al.* (2006) speculate that *H. pylori* can create an inflammation in the middle ear by a similar way like that described in the stomach mucosa.

CONCLUSION

Although, there are a high positivity rate of *H. pylori* infection by CLO test (60%) and 14C UBT (64.3%) of patients with CSOM, there only a few positivity rates by immunohistochemical study (7.5%). This may be due to lack of presence of the organism in tissues or due to difficult identification due to small tissue specimen. This results does not support a role of *H. pylori* infection in pathogenesis of CSOM. Further studies are needed to confirm the presence of the organism in middle ear cleft and its possible role in pathogenesis of CSOM.

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الملخص العربي**الكشف عن بكتريا الهيليكوباكتر بايلورى فى الاذن الوسطى ودورها فى التهاب الاذن الوسطى الصديدي المزمن**

نظرا للعلاقة الوثيقة بين الارتجاع المعدي المريئي والتهابات الاذن الوسطى فان وجود جرثومة هيليكوباكتر بايلورى فى الاذن الوسطى ودورها المحتمل فى تفسير التهاب الاذن الوسطى المزمن يحتاج للدراسة. لهذا اجريت هذه الدراسة على 40 مريضا (25 من الذكور و 15 من الاناث) فى مستشفيات جامعة الازهر فى الفترة من يونيو 2014 الى يونيو 2016. وتراوحت اعمار المرضى بين 18 الى 58 سنة. اما المجموعة الضابطة فتكونت من 20 شخصا تراوحت اعمارهم بين 19 الى 29 سنة. وتم اخذ موافقة كتابية من المرضى والمجموعة الضابطة. وتم عمل اختبار CLO, واختبار 14C UBT لكل المرضى كما تم عمل اختبار هستوكيميائى مناعى لكل العينات باستخدام مضادات بكتريا الهيليكوباكتر. وكانت النتائج لحالات اختبار CLO ايجابى فى 24 مريضا (60%). اما اختبار 14C UBT فكان ايجابيا فى 26 مريضا (65%). وكانت هناك دلالة احصائية بين اختبار CLO واختبار 14C UBT للمرضى ($P < 0.002$). اما اختبار 14C UBT للمرضى والمجموعة الضابطة فكان هناك فارق احصائى ($P < 0.001$). اما بالنسبة للفحص هستوكيميائى مناعى فلم يعطى نتائج ايجابية الا فى 3 حالات فقط. ونستخلص من هذه الدراسة انه لم يتأكد وجود دور لبكتريا الهيليكوباكتر بايلورى فى تفسير حدوث التهاب الاذن الوسطى المزمن ونحتاج لدراسات اخرى لمعرفة تأثير هذه البكتريا ودورها فى حدوث التهاب الاذن الوسطى المزمن.