THE STUDY OF PREVALENCE OF CAG A (+VE) FACTOR WHICH IS ONE OF VIRULENCE FACTORS OF H.PYLORI AMONG GASTRIC CANCER PATIENTS

Thesis

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وأنزل الله عليك
الكتاب والحكمة
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تَعْلَمُ وكَانَ فَضْلُ اللَّهِ
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صدق الله العظيم
سورة النساء الآية
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List of Abbreviations

ALP	Alkaline phosphatase
ALT.....	Alanine transaminase
AST	Aspartate transaminase
Bid	Twice per day
BMDCs.....	Bone marrow derived cells
BMT.....	Bismuth subsalicylate, metronidazole, and tetracycline
Cag A	Cytotoxin associated gene antigen
CD	Cluster of differentiation
COX2	Cyclooxygenase 2
CI	Confidence interval
CSC	Cancer stem cell
CT	Computed Tomography
DNA	Deoxyribonucleic acid
ECF	Epirubicin, cisplatin and 5-Fluorourasil
ECOG	Eastern Cooperative Oncology Group
EGD	Esophagogastroduodenoscopy
EMR	Endoscopic mucosal resection
FBC	Full blood count
5-FU	5-Fluorourasil
GC	Genome content OR Gastric Cancer
GEP.....	Gastric progenitor cells
GGT.....	Gamma glutamyl transferase
HB	Hemoglobin
HCV.....	Hepatitis C virus
HIV.....	Human immunodeficiency virus
HOP	Helicobacter outer membrane porins
Hor	Hop-related proteins
IFN	Interferone
IL	Interleukin
ITT	Intention-to-treat
LA	Lansoprazole and amoxicillin

List of Abbreviations

LAL	Levofloxacin, amoxicillin, and lansoprazole
LAC	Lansoprazole, amoxicillin, and clarithromycin
LFTs	Liver function tests
Lgr5	Leucine-rich repeat-containing G protein-coupled Receptor 5, Gpr49
LPS	Bacterial lipopolysaccharide
MAPK.....	Mitogen –activated protein kinases
MALT	Mucosa associated lymphoid tissue
M RNA.....	Messenger Ribonucleic acid
MSCs.....	Mesenchymal stem cells
Msi-1.....	Musashi-1
NICE.....	National Institute for Health and Clinical Excellence
NFAT.....	Nuclear factor of activated T-cells
NSAID.....	Non steroidal anti inflammatory drugs
OAC.....	Omeprazole, amoxicillin, and clarithromycin
OR.....	Odds ratio
PI3K.....	Phosphatidylinositol 3-kinase
qd.....	Four times per day
RNA.....	Ribonucleic acid
Rr	Relative risk
SCID	Severe combined immunodeficiency

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ABSTRACT

Gastric cancer is currently the second most common cause of cancer death in the world and the fifth most common cancer and the fourth leading cause of cancer related death in Europe. It has been evident for over 20 years that H. pylori is involved in the development of gastric adenocarcinoma . The cagA gene of H. pylori is the main virulence factor that leads to the development of gastric adenocarcinoma through the derangement of cellular architecture and signaling. The objective of our work is to study the prevalence of Cag A amoung Gastric cancer patients.

This descriptive study was done on 60 gastric cancer, at the age group of 22 to 78 years old. They were 30 males and 30 females.All patients were subjected to full history taking, complete clinical examination, laboratory investigations including Hemoglobin, Plt, Tlc, Albumin, ALT, AST, GGT, ALP, Total&Direct bilirubin, Urea, Creatinine, Na, k, Ca, Phos, Uric acid.

H.pylori work up was done by Serum samples were provided from the patients to be tested to detect Anti H pylori IgM and Anti CagA IgG.Serum samples will be stored at −80°C until serological testing will be performed using (Helicobacter pylori line IgA/IgG immunoblot GB

Also all the patients done Computerized tomography (CT) scan of abdomen & pelvis with contrast and Upper endoscopy and biopsy taking and if needed CT guided biopsy followed by histopathological examination.

Our results showed that 34 patients(56.67%) are Cag A +ve and 26 patients(43.33%) are Cag A –ve with no statistically significant difference as regard the gender or age.

We concluded that Cag A play significant role in development of gastric cancer in patients who were infected by H pylori.

Keywords: Gastric cancer, H.pylori, CAG A, Incidence of gastric cancer in eastern and western countries.

INTRODUCTION

Gastric cancer is currently the second most common cause of cancer death in the world and the fifth most common cancer and the fourth leading cause of cancer related death in Europe. In Egypt the frequency of gastric cancer is 2% in males and 1.5% in females from the newly diagnosed cases with median age of 54 ***(National Cancer Institude of Egypt website, 2005)***.

Gastric cancer is the 3rd most common cancer site for males and 5th for females. It accounts for about 9% of all cancer worldwide.Gastric cancer much more common in certain asian, central Europe, central and south American countries especially japan, chille, costa rica, hungary and Poland.

It has been evident for over 20 years that H. pylori is involved in the development of gastric adenocarcinoma; in 1994, the WHO concluded that *H. pylori* is a definite or class I carcinogen in humans. H. pylori is responsible for about 75% of all noncardia gastric cancers and 63.4% of all stomach cancers worldwide ***(Wroblewski et al., 2010)***.

A strain specific H. pylori gene cagA (cytotoxin associated gene A) is a component of the cag Pathogenicity Island. Several genes within this island encode products that are homologs of proteins of the type IV bacterial secretion pathway.The cagA gene of H. pylori is the main virulence factor that leads to the development of gastric adenocarcinoma through the derangement of cellular architecture and signaling ***(Hatakeyama, 2008).***

The *cag* (cytotoxin-associated gene) pathogenicity island (*cag* PAI) is a 40 kilobase segment of DNA, containing 31 genes, many of which encode components of a type 4 bacterial secretion system. The secretion system acts as a molecular syringe for delivery of bacterial products, including the cag gene product CagA and peptidoglycan component into eukaryotic cells. The *cag* PAI plays an important role in H. pylori pathogenesis, and is not expressed in all strains. Approximately 60% of *H. pylori* strains isolated in Western countries carry the *cag* PAI, whereas almost all of the East Asian strains isolated are *cag* PAI-positive ***(Backert et al., 2010).***

H. pylori CagA and peptidoglycan (PGN)are injected into host cell cytoplasm following its adherence to gastric epithelial cells, and induce CagA- and *cag*PAI-dependent effects on epithelial cells. ***(Viala et al., 2004)***.

Aim of the work

So, the aim of this study is to determine the prevalence of CAG A virulence factor in gastric cancer patients infected with H-pylori, to examine the association of CAG A virulence factor of H-pylori with gastric cancer and to prove the increased incidence of gastric cancer in patients infected with H-pylori strains which have cag A virulence factor.

Understanding this relationship may provide valuable data for understanding gastric carcinogenesis and for development of strategies to treat H-pylori and screen for gastric cancer in patients infected with H-pylorus.

Chapter *I*

H. PYLORI

Introduction

H. pylori previously named Campylobacter pyloridis, is a gram-negative, microaerophilic bacterium found in the stomach. It was identified in 1982 by Barry Marshall and Robin Warren, who found that it was present in patients with chronic gastritis and gastric ulcers, conditions that were not previously believed to have a microbial cause. It is also linked to the development of duodenal ulcers and stomach cancer. However, over 80 percent of individuals infected with the bacterium are asymptomatic and it has been postulated that it may play an important role in the natural stomach ecology ***(Blaser, 2006).***

More than 50% of the world's population harbor H. pylori in their upper gastrointestinal tract. Infection is more prevalent in developing countries, and incidence is decreasing in Western countries. H. pylori's helix shape (from which the generic name is derived) is thought to have evolved to penetrate the mucoid lining of the stomach ***(Yamaoka, 2008).***

In recognition of their discovery, they were awarded the 2005 Nobel Prize in Physiology or Medicine ***(Nobelprize.org medicine laureates, 2005).***

Scientific classification

Domain: Bacteria Phylum: Proteobacteria Class: Epsilonproteobacteria

Order:CampylobacteralesFamily:HelicobacteraceaeGenus:Helicobacter

Species:H. pylori

Binomial name

Helicobacter pylori

(Marshall et al., 1985; Goodwin et al., 1989)

Microbiology

H. pylori is a spiral shaped, microaerophilic, gram negative bacterium measuring approximately 3.5 microns in length and 0.5 microns in width. In vitro, it is a slow growing organism that can be cultured on blood agar or selective media such as Skirrow's media incubated at 37ºC in a 5 percent oxygen atmosphere for three to seven days. Small, uniformly sized, translucent bacterial colonies form and the organisms can be morphologically characterized by Gram stain and their typical spiral or rod shaped appearance. High power microscopy reveals that the organism has two to seven unipolar sheathed flagella which enhance its mobility through viscous solutions.

If the growth environment is less than ideal, coccoid forms of H. pylori can occasionally be seen in culture. These coccoid forms are thought to represent an adaptation to hostile surroundings; they appear to be more resistant and may enable the organism to survive for periods of time outside the human host in feces or in drinking water.

In addition to morphologic characterization, the organism can be biochemically characterized as catalase, oxidase, and urease positive. Urease appears to be vital for its survival and colonization; it is produced in abundance, making up more than 5 percent of the organism's total protein weight. Bacterial urease activity is clinically important because it forms the basis for several invasive and noninvasive tests to diagnose infection.

(Uptodate, 2012)

Genome

H. pylori consists of a large diversity of strains, and the genomes of three have been completely sequenced.The genome of the strain "26695" consists of about 1.7 million base pairs, with some 1, 550 genes. The two sequenced strains show large genetic differences, with up to 6% of the nucleotides differing.

(Helicobacter, 19 99; Complete genome" National Center for Biotechnology, 2008).

Study of the H.pylori genome is centered on attempts to understand pathogenesis, the ability of this organism to cause disease. Approximately 29% of the loci are in the "pathogenesis" category of the genome database. Two of sequenced strains have an approximately 40 kb-long Cag pathogenicity island (a common gene sequence believed responsible for pathogenesis) that contains over 40 genes. This pathogenicity island is usually absent from H. pylori strains isolated from humans who are carriers of H. pylori, but remain asymptomatic***. (Baldwin et al., February, 2007).***

The cagA gene codes for one of the major H. pylori virulence proteins. Bacterial strains that have the cagA gene are associated with an ability to cause ulcers.The cagA gene codes for a relatively long (1186 amino acid) protein. The cag pathogenicity siland (PAI) has about 30 genes, part of which code for a complex type IV secretion system. The low GC-content of the cag PAI relative to the rest of the Helicobacter genome suggests the island was acquired by horizontal transfer from another bacterial species***. (Amieva et al., January 2008).***

Epidemiology

In developed nations it is currently uncommon to find infected children, but the percentage of infected people increases with age, with about 50% infected for those over the age of 60 compared with around 10% between 18 and 30 years. The higher prevalence among the elderly reflects higher infection rates when they were children rather than infection at later ages. In the United States, prevalence appears to be higher in African-American and Hispanic populations, most likely due to socioeconomic factors.The lower rate of infection in the West is largely attributed to higher hygiene standards and widespread use of antibiotics. Despite high rates of infection in certain areas of the world, the overall frequency of H.pylori infection is declining.However, antibiotic resistance is appearing in H. pylori; there are already many metronidazole- and clarithromycin-resistant strains in most parts of the world ***(Malaty, 2007)***.

Mode of transmission

H. pylori is contagious, although the exact route of transmission is not known.Person-to-person transmission by either the oral-oral or fecal-oral route is most likely. Consistent with these transmission routes, the bacteria have been isolated from feces, saliva and dental plaque of some infected people. Findings suggest that H. pylori is more easily transmitted via gastric mucus than via saliva.Transmission occurs mainly within families in developed nations yet can also be acquired from the community in developing countries.H. pylori may also be transmitted orally by means of fecal matter through the ingestion of waste-tainted water, so a hygienic environment could help decrease the risk of H. pylori infection ***(Delport and van, 2007)***.