Detection of Helicobacter Species in liver cirrhosis with and without Hepatitis (C) Virus Infection

Thesis

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بِسْمِ اللّهِ الرّحمَنِ الرّحيمِ

[...رَبِّ أَوزِعنِي أَن أَشكُرَ نِعمَتَكَ
الَّتِي أَنْعَمْتَ عَلَيَّ وعَلى والِدَيَّ
وأَنْ أَعْمَلَ صَالِحاً تَرْضَاهُ
وأَدْخِلْنِي بِرَحْمَتِكَ فِي
عِبَادِكَ الصَّالِحِينَ]

صدق الله العظيم

النمل.. اية رقم ١٩

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List of Abbreviations

AFP : Alpha fetoprotein

Cdt : Cytolethal Distending Toxin

CLO : Campylobacter-like organism test

CLSI : Clinical and laboratory standard institute

CPI : Cag Pathogenicity Island

EGF : Epidermal growth factor

EGFR : Epidermal Growth Factor Receptor

EIA : Enzyme Immuno Assay

HBV : Hepatitis B virus

HCV : Hepatitis C virus

HCC : Hepatocellular carcinoma

HIV : Human immunodeficiency virus

INF-γ : Gamma interferon

ITP : Idiopathic thrombocytopenic purpura

MALT : Mucosa associated lymphoid tissue

MIC : Minimal inhibitory concentration

MMP : Matrix Metallo Proteinases

PCR : Polymerase Chain reaction

PUD : Peptic ulcer disease

RFLP : Restriction Fragment Length Polymorphism

RUT : Rapid urease test

TK : Tyrosine Kinase

UBT : Urea breath test

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Introduction

Hepatocellular carcinoma (HCC) was the fourth cause of cancer death worldwide ***(Moushira et al., 2011)***. This tumour often follows chronic inflammation and long standing cirrhosis ***(Pellicano et al., 2004)***.

Cirrhosis of the liver is a diffuse process, characterized by fibrosis and nodule formation, which stems from hepatocellular injury. The cellular necrosis might originate from viral, toxic, metabolic and autoimmune sources. There are extensive data indicating that chronic infection can lead to cancer in various organs. Parasites such as schistosomes and liver flukes, and a bacterium, Helicobacter pylori, were classified as type I liver carcinogens by the International Agency for Research on Cancer in 1997. The most competing evidence comes from hepatocellular carcinoma (HCC) where chronic hepatitis B virus (HBV) infection is considered to be direct etiological factor and hepatitis C virus (HCV) infection a major risk factor for this disease ***(Pellicano et al., 2004; Rocha et al., 2005)***.

Chronic inflammation perse is known to be a factor of progression towards cancer ***(Cassel, 1998)***, though HCV itself does not appear to foster a strong inflammatory mechanism. It is thus not yet clear what factor most influence the outcome of the

liver disease. Searching for other noxae has therefore become mandatory ***(Mosnier et al., 1994)***.

Helicobacter infections have been implicated in the occurrence of certain liver diseases in some animal species such as Helicobacter canis in dogs, Helicobacter hepaticus and Helicobacter bilis in mice. In humans, Helicobacter pylori DNA has been detected in the liver of patients suffering from cholestatic diseases and HCC arising from non cirrhotic liver ***(Pellicano et al., 2004; Shi et al., 2006)***.

A study suggested that, presence of Helicobacter species in liver samples could possibly serves as a cofactor in the development of end-stage of liver diseases in humans. These concern have spurred considerable interest in determining the mechanism by which these extracellular bacteria and the associated inflammatory response endorse hepatic and biliary disease ***(Santosh et al., 2006)***.

Studies showed a high frequency of *H.pylori* and *H.pullorum* detected by PCR in the liver of patients with cirrhosis and superimposed HCC with and without associated HCV infection, suggesting that, these bacteria might play an important role in the progression of chronic HCV patients to cirrhosis and HCC ***(Rocha et al., 2005)***.

The mechanism by which *H. pylori* colonizes the human liver is not totally enlightened. The *H. pylori* DNA detected in the liver tissue may result from bacterial translocation from the stomach into the blood through the portal system, especially in the later stage of chronic liver disease when portal hypertension occurs. In addition, the bacteria may reach the liver by phagocytes and macrophages or circulating retrograde transfer from the duodenum. Several researchers have suggested that H. pylori may damage hepatocyte by a cytopathic effect ***(Jose et al., 2014).***

Aim of the Work

The aim of the present study is to determine the presence of Helicobacter species in the liver of cirrhotic patients, with or without HCV infection.

Helicobacter

Historical Background:

Gastric spiral shaped bacteria have been observed in animals and humans for more than 100 years. The first recorded observation of gastric spiral-shaped bacteria in animals was made by Rappin in 1881 and Bizzozero in 1893. The first observation in human was made by Krienitz in 1906 ***(Fox and Megraud, 2007)***.

In 1982, Campylobacter pyloridis (later known as Helicobacter pylori) was successfully cultured from stomach biopsy specimens from human patients with gastritis. Subsequently, other spiral gram-negative bacteria have been observed in and isolated from the gastrointestinal tract of mammals such as cats, dogs and rodents ***(Fox et al., 2000)***.

Taxonomy:

Initially, many spiral gram-negative bacteria isolated from the mammalian gastrointestinal tract were grouped as Campylobacters. This was based on similar microscopic and ultrastructural morphologies, common microaerobic growth requirements, and similar ecologic niches. However, following extensive analysis of enzymatic activities, fatty acid profiles, 16S rRNA sequence analysis, nucleic acid hybridization and 23S rRNA analysis, the genus Helicobacter was formally distinguished from Campylobacter ***(Megraud et al., 2012)***. The Helicobacter genus belongs to class Epsilonproteobacteria, order

campylobacterales, and Helicobacteraceae family, and already involve more than 35 species**.** *Helicobacter Pylori* is of primary importance for medicine, however, non-*pylori* Helicobacter species have been detected in human clinical specimens and showing different organ specificity ***(Boyanova, 2014).***

Clinically Significant Helicobacters:

A- Enterohepatic Helicobacter species:

These Helicobacter have been identified in liver specimens from patients with primary sclerosing cholangitis, cholangiocarcinoma, liver cirrhosis, and hepatocellular carcinoma associated with hepatitis C virus infection by genus specific PCR amplification and partial DNA sequencing ***(Fox and Megraud, 2007)***. It has been suggested that the presence of these Helicobacter species in liver samples could serve as a co-factor in the development of end stage of liver disease ***(Pellicano et al., 2008).***

1. Helicobacter hepaticus (H.hepaticus):

It was isolated from liver, cecal and colonic mucosa of mice with chronic active hepatitis ***(Rocha et al., 2005)***.

H.hepaticus known to cause chronic active hepatitis and is associated with multifocal necrotic hepatitis in many strains of mice, and is responsible for the development of hepatic adenoma and hepatocellular carcinoma in mice ***(Santosh et al., 2006)***. This organism has been isolated from human and has