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CURRENT STUDIES ON MICROBIAL AND BIOCHEMICAL ALTERATIOINS IN PEPTIC ULCER, GASTRITIS AND CERVICAL CANCER IN MALE AND FEMALE WISTER RATS

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ABSTRACT

Malignant neoplasm is a class of disease in which a group of cells display the trait of uncontrolled growth, invasion and metastasis. Cancer is a leading cause of death worldwide. Peptic ulcer (stomach cancer), gastritis and cervical cancer is mainly caused by selected strains of Helicobacter pylori and Papilloma virus. Colonization of *Helicobacter pylori* in stomach lumen and upper part of small intestine results in chronic Gastritis and peptic ulcer. Papilloma virus is suggested to cause the cancer of cervix. Free radicals are also responsible for the development of various types of cancer. The present study mainly focuses on establishing the role of free redicals in the development of peptic ulcer and cervical cancer along with *H. pylori* and Papilloma virus.

KEYWORDS: *Helicobacter pylori*, Papiloma virus, Peptic ulcer, Gastritis, Cervical cancer.

INTRODUCTION

Cancer or malignant neoplasm is a class of disease in which a group of cells display the trait of uncontrolled growth, invasion, and metastasis. According to American cancer society, 7.6 million people died from cancer in the world during 2007 and responsible for about 13% of all deaths. Peptic ulcer or gastritis (stomach cancer) is commonly caused by selected strains of *Helicobacter pylori*. Other common risk factors include smoking, alcohol consumption, excessive NSAID consumption etc.

H. pylori (*Campylobacter pylori*) are gram negative, microaerophilic bacterium found in stomach. It was identified by Barry Marshal and Robin Warren who found that it was present in patients with chronic gastritis and gastric ulcer. It is also linked to development of duodenal ulcer and stomach cancer. However, 8% of individual infected with bacterium are asymptomatic and it has been postulated that it may play role in the natural stomach ecology.

H. pylori are helix shaped, gram negative about 3 micron meters long with a diameter of about 0.5 micron meter. It produces oxidase, catalase and urease. It consists of long diversity of strains and the genome of 3 strains has been completely sequenced. Two sequenced strains have an approximately 40 Kb long CAG pathogenicity island (a common gene sequence believed responsible for pathogenesis).

Besides *H. pylori* infection, oxidative stress may also play role in the development of disease. Oxidative stress may be defined as a shift in balance in cellular oxidation-reduction in favor of oxidation, which leads to damage to the cells and information of molecular products that are indicators of oxidative stress. Free radicals and reactive oxygen species are neutralized by antioxidant system and protect the biological tissues from damage due to free radicals. The antioxidant system includes numerous enzyme and non-enzyme type of antioxidant group that are located in the cells and extracellular fluid. The antioxidant enzymes are Superoxide dismutase, Catalase, and Glutathione peroxidase. The non-enzymatic are Vitamin C and E. Cancer of cervix is a malignancy of cervix or cervical area. It is fifth most common cancer in human and the second most common cancer found in women after breast cancer. The risk factors for cervical cancer are viral infections (HPV, HIV, and HSV), multipartity, early initiation of sexual activity, multiple sex partenrs, smoking, low antioxidant diet, poor hygiene etc. The present study mainly focuses on evaluation of Antioxidant enzyme levels in *H. pylori* induced gastric ulcer in male wister rats and preinduced cervical cancer in female wister rats.

MICROBIOLOGY OF *Helicobacter Pylori* BACTERIA

Helicobacter pylori, previously called *Campylobacter pyloridis*, are gram negative microaerophilic bacterium found in 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. It is also linked to the development of duodenal ulcers and stomach cancers. However, over 80% of individuals infected with bacterium are asymptomatic and it has been postulated that it may play role in the natural stomach ecology. 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 are a helix shaped, gram negative bacteria, about 3 micrometers long with a diameter of about 0.5 micrometers. It is microaerophilic, i.e. it requires oxygen but at low concentration than atmosphere. It contains hydrogenase which can be used to obtain energy by oxidizing molecular hydrogen produced bby intestinalo bacteria. It is capable of forming biofilms. It produces enzymes like Oxidase, Catalase and Urease. It can convert from Spiral to a possibly viable but non-culturable, coccoid form.

H. pylori consist of large diversity of strains, and the genomes of 3 have been completely sequenced. The genomes of the strain "26695" consist of about 1.7 million base pairs with some 1,550 genes. The two sequenced strains show large genetic differences, with up 6% of the nucleotide differing. Study of *H. pylori* genome is centered on attempt 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 and approximately 40 Kb long Cag pathogenicity island (a common gene sequence believed responsible for pathogenesis).

PATHOPHYSIOLOGY OF PEPTIC ULCERS, GASTRITIS AND CERVICAL CANCER

To colonize the stomach, *H. Pylori* must survive the acidic pH of lumen and uses its Flagella to borrow into the mucus to reach its niche, close to the stomach's epithelial cell layer. Many bacteria can be found deep in the mucus, which is continuously secreted by mucous secreting cells and removed on the lumen side. To avoid being carried into lumen, *H. pylori* sense the pH gradient within mucous layer by chemotaxis and swims away from the acidic content of lumen towards more neutral pH environment of the epithelial cell surface. *H. pylori* are also found inner surface of the stomach epithelial cells and occasionally inside epithelial cells. H. pylori produces large amount of enzyme Urease, molecules of which are localized inside and outside of the bacterium. Urease breaks down Urea (which is normally secreted in the stomach) to carbon dioxide and Ammonia. The Ammonia is converted to Ammonium by accepting a proton (H^+), which neutralizes gastric acid. The survival of *H. pylori* in acidic stomach is dependent on urease. Colonization of stomach by *H. pylori* results in chronic Gastritis, an inflammation of the stomach lining. Duodenal and Stomach Ulcers results when the consequences of inflammation allow the acid and pepsin in the stomach to overwhelm the

mechanisms that protect the stomach and duodenal mucosa from these caustic substances. The type of ulcer that develops depends on the location of chronic gastritis, which occurs at the site of *H. pylori* colonization. The acidity within the stomach lumen affects the colonization pattern of *H. pylori* and therefore ultimately determines whether a duodenal or gastric ulcers will form. The inflammatory responses to the bacterium induce G-cells in the Antrum to secrete hormone Gastrin which travels through the blood stream to Corpus. Gasrin stimulates the parietal cells in the Corpus to secrete more acid in the stomach lumen. Chronically increased Gastrin level eventually causes the number parietal cells to also increase, further escalating the amount of acid secreted. The increased acid load damages the duodenum and ulceration may eventually result. In contrast, gastric ulcers are often associated with normal or reduced gastric acid production suggesting the mechanisms that product the gastric mucosa are defective. However, chronic inflammation induced by bacteria causes' further reduction of acid production and eventually Atrophy of stomach lining which may lead to gastric ulcers and increases the risk of stomach cancer.

Cancer of cervix is a malignancy of cervix or cervical area. It is the fifth most common cancer in human and the second most common cancer found in women after breast cancer. The risk factors for cervical cancer are viral infections (HPV, HIV, and HSV), multipartity, early initiation of sexual activity, multiple sex partners, smoking, low antioxidant diet, poor hygiene etc. Recent studies revealed the role of oxidative stress in cervical cancer. Oxidative stress may be defined as a shift in balance in cellular oxidation-reduction reaction in favor of oxidation, which leads to damage to the cells and formation of molecular products that are indicators of oxidative stress. The free radicals and reactive oxygen species are neutralized by antioxidant system and protect the biological tissues from damage due to free radicals and can be recycled or regenerated by biological reducers. The antioxidant system includes numerous enzymes and non-enzyme types of antioxidant groups that are located in the cells and extracellular fluid. The antioxidant enzymes are superoxide dismutase (SOD), catalase, and Glutathione peroxides. The non-enzymatic are Vitamin C and E.

Super oxide dismutase is a class of closely related enzymes that catalyzes the breakdown of highly reactive superoxide anion into oxygen and hydrogen peroxide. Catalase is a cytosolic enzyme containing four iron atoms in a 500 amino acid protein.

Catalase catalyzes the conversion of hydrogen peroxide to water and oxygen. Glutathione peroxide is an enzyme with four selenium containing groups that catalyze the breakdown of hydrogen peroxide and protect the lipid in cell walls from per oxidation.

Vitamin E is a fat soluble vitamin and one of a number of nutrients called antioxidant. Vitamin C is another well-known antioxidant. Both vitamin C and vitamin E can protect against cancer. Both are highly effective antioxidant. It can protect indispensable molecules in the body such as proteins, lipids, carbohydrates, and Nucleic acids from damage by free radicals and reactive oxygen species that can be generated during normal metabolism as well as through exposure to toxins and pollutants. Some studies suggest that diets rich in antioxidants, including vitamin E and C may be connected to reduced risk of carefully it has been found that though work has been conducted in establishing the role of antioxidants in the prevention and management of various types of cancers, but much has yet to be explored regarding the role of natural antioxidant in the management of cervical cancer, Peptic ulcer and Gastritis. The present study also aims at exploring the role of various strains of H. pylori bacterium in the development of Peptic ulcers and Gastritis.

RECENT STUDIES

Cervical cancer is a malignant neoplasm arising from uterine cervix. About 80% of cervical cancers are of the squamous type, the reminders are adenocarcinomas, adenosquaqmous carcinomas and other rare types (Waggoner SE, 2003). Cervical cancer represents the second most common cancer in women worldwide. Cervical cancer is preceded by skin cancer and breast cancer as the most common cause of death worldwide. Cervical cancer is preceded by skin cancer and breast cancer as the most common cause of death worldwide (Riesetal, 2001). Oxidative stress is potentially harmful to cells, and reactive oxygen species (ROS) have been known to play an important role in initiation and promotion of multi-step carcinogenesis (12-Chen et al. 2000). The imbalance between the pro-oxidants and antioxidants in favor of pro-oxidants is called oxidative stress. (M. Smita K. Naidu et al. 2007).

Some studies show that the oxidative stress markers have apparent association in the development of cervical cancer and related complications. (Notani PN etal. 2001, M. Smita, K. Naidu et al. 2007).

Many researchers have shown the potential of ROS to damage DNA causing mutations. It is evident that various virus infections and ROS have same effect (M. Smita, K. Naidu etal, 2007); therefore the role of ROS in cancer is to be studied.

Superoxide dismutase mainly functions to provide a defensive action against the potentially damaging relativities of superoxide radicals generated by all aerobic metabolic reactions. As superoxide dismutase is a free radical metabolizing enzyme, it catalyzes the dismutation of superoxide radicals to hydrogen peroxide. This protects the cell membrane from damage by reactive oxygen species. But the decreased superoxide dismutase level may lead to increased lipid per oxidation resulting in the cellular rigidity and deformability (Yamanaka et al., 2004). Alterations in superoxide dismutase activity in cervical cancer patients have been revealed recently (Manoharan et al., 2004).

Glutathione peroxides are selenium dependent antioxidant enzyme which catalyzes detoxification of hydrogen peroxide and other through glutathione. The integrity of cervical cell can be presumed to be maintained by it. Alterations in glutathione peroxides activity in cervical cancer patients have been found (Rohini C Sane et al 2006).

Vitamin-C (ascorbic acid) is a water soluble antioxidant vitamin. Its role as an antioxidant is indicated by its known free radical scavenging action. As a reducing agent and antioxidant agent it directly reacts with oxygen and hydroxyl free radicals and various lipid hydro peroxides. So, it may be said that role of Vitamin-C is very beneficial in cervical cancer treatment (Niki et al, 1991; koechlin et al, 1998).

Vitamin-E (alpha tocopherol) is an antioxidant that assists in maintaining cell integrity. The statistically significantly lower level of alpha-tocopherol was observed in blood serum of cervical intraepithelial neoplwasia patient.

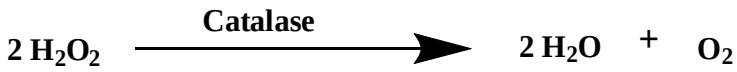
Several studies revealed the role of *H. pylori* bacterium in the development of duodenal ulcer, peptic ulcer and Gastritis (R J Hopkins, L S Giradi, E A Turney, 1996). Oxidative stress is believed to initiate and aggravate peptic ulcers and gastric carcinoma. An increase in serum lipid peroxide and tendency to decrease superoxide dismutase and catalase levels were observed in infected rats. Some studies show a positive correlation between free radical induced oxidative stress in gastric and duodenal ulcers and gastric carcinoma (Tandon R, Khanna H D, Dorababu M, Goal R K. 2004).

PLAN OF WORK

a. Male and Transgenic female wister rats were used weighing 180 to 200gm.
b. The rats were divided into three groups, each group containing six rats. The first group is control group; second group is of male wister rats infected with *H. pylori.* The third group is of transgenic female wister rats which have pre-induced cervical cancer.
c. The levels of Catalase and Glutathion peroxidase were evaluated and compared.
d. The Biochemical parameters were analyzed by the following procedures.

Catalase

CAT is a ubiquitous heme protein that reduces H_2O_2 to water. Hence catalase activity was determined by measuring decreasing absorbance of hydrogen peroxide.

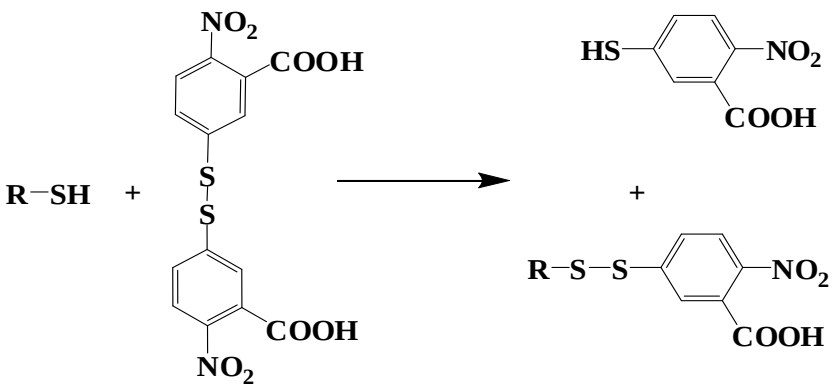


Procedure

100 µL of supernatant of ulcer tissue homogenate was added to cuvette containing 1.9 mL of 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0 mL of freshly prepared 30 mM H_2O_2. Decrease in absorbance was read at 240 nm for 3min at interval of 30 sec. The activity was calculated using extinction coefficient of H_2O_2 0.041 µM/cm^2. Results were expressed as micromole of H_2O_2 utilized/min/gm tissue. The rate of Decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm mentioned by Sinha *et al.*, (1972).

Reduced glutathione

The procedure is based on method of Ellman, that 5, 5'-dithiobis-(2-nitrobenzoic acid) is reduced by SH groups of glutathione to form 1 mole of 2-nitro-5-mercaptobenzoicc acid per mole of SH. The nitromercaptobenzoic acid anion has an intense yellow color and can be used to measure SH groups. The reaction is:



Procedure

The supernatant (40µL) was mixed with 400µL tris and 360µL water. Then 0.2 mL (200µL) DTNB solution was added and absorbance was measured at 412 nm. Standard curve for GSH was prepared using glutathione by Ellman *et al.*, (1959).

CONCLUSION

Oxidative stress is of concern to clinicians, managing the cancer patients, but with judicious dosing and proper supplementation of antioxidant the complications of Gastritis, Peptic ulcer and cervical cancer may be minimized. The present study shows that there is marked reduction in antioxidant levels in wister rats infected with *H. pylori* and the female rats with pre-induced cervical cancer. The study suggest that diet rich in antioxidants and vitamins can be helpful in the proper management of various types of malignant cancers. The presented information will help the clinicians in understanding the importance of antioxidants and better management of their patients. The ongoing studies focus on the fact that whether *H. pylori* eradication may be helpful in minimizing the complications of peptic ulcers and gastritis.

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