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CLINICAL PATHOLOGIC STUDY OF ORAL ADMINISTRATION OF *HELICOBACTER PYLORI* IN RAT

REZA RANJBAR[1], FARZAMDELDAR[2] AND KAVEHA ZIMZADEH[3]

1: Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

2: Graduate of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran

3: Department of Clinical Sciences, Veterinary Faculty, Urmia Branch, Islamic Azad University, Urmia, Iran

ABSTRACT

In recent study, we aimed to evaluate whether some plasma parameters are altered after oral administration of of *Helicobacter pylori* in rat. *H pylori* is known to be as the most common agent in peptic ulcer and chronic gastric incidence. For this purpose, twelve Albino male wistar rats were assigned into two groups and after ten days acclimatizing, 1ml prepared suspension of *H pylori* (0.5 Macfarland tube, $1/5\times10^8$)were administrated by gavage to each of rats (treatment group) and control group received same amount of distilled water. After three month, plasma total sialic acid (TSA), malondialdehyde (MDA), hepcidin (Hep), homocysteine (Hcy), sphingosine 1 phosphate (S1P), adenosine deaminase (ADA), copper(Cu) and zinc (Zn) were measured. The results indicated increase of all parameters in treatment group rather than healthy ones.

In conclusion, the elevated plasma parameters suggested the impact of *H pylori*upon these ones in rat and it seems that more investigation needs to detectthe possible biochemical biomarker among them.

Keywords: Helicobacter Pylori, Sialicacid, Malondialdehyde, Hepcidin, Homocysteine, Sphingosine 1 Phosphate, Adenosine Deaminase, Copper, Zinc

INTRODUCTION

The most common chronic bacterial infection in humans belongs to *Helicobacter pylori* (*H pylori*) infection. It is known to be as the main etiologic factor in the induction of chronic gastritis, peptic ulcer and gastric cancer **(Lopez-Saez *et al*., 2010; Hosseinzadeh *et al* 2011)**. H. pylori infection (in 2% cases) causes in 65–80% of gastric cancers **(You *et al*., 2000; Testerman *et al*., 2006; Vilchis *et al*., 2009; Janjetic *et al*., 2010; Zamani *et al*., 2011; zhang *et al*., 2012; Izadi *et al* 2012)**. Several studies have explained *H pylori* related serum parameters alterations such as trace elements, but the wide varieties of them have not been determined. (**Zhang *et al*., 2012; Janjetic *et al*.,2010., Vijayan *et al*., 2007; Navvabi *et al*., 2013; Rasool *et al*., 2012; Ozer *et al*., 2005)**. Hence, this is the first work evaluation ofclinical pathologic studies of *H pylori* infection.

Adenosine is noticed an essential endogenous inhibitor of the immune system which its potent suppressor activity has virtually been determined in all cells of immune system and its levels are regulated by adenosine deaminase (ADA, E.C. 3.5.4.4). ADA involves in degradation of adenosine and deoxyadenosineto inosine and deoxyinosine. ADA as a substantial enzyme contributes in the maturation and differentiation of T lymphocytes and macrophage monocyte system as well as its activity is higher in T cells than B cells. It regulates the cellular mechanisms associated with blood flow, vasodilatation, angiogenesis, proliferation and its activity has been determined higher in diseases with immune response stimulation such as liver cirrhosis, chronic hepatitis and hepatocellular carcinoma. Although the enhancement of ADA activity is usually determined in tuberculosis but also can be observed in the other diseases (infectious or non-infectious) such as typhoid fever, sarcoidosis, and acute lymphoblastic leukemia.

Hcy, as a sulfur-containing amino acid, is generated from the intracellular demethylation of methionine. It is involved in endothelial cell damage in experimental animals and cardiovascular diseases in human. Hyperhomocysteinemia participates in oxidative stress occurrence and plays major role in pathological effects, which has been demonstrated as a mechanism involved in the formation of anaemia. Moreover, hyperhomocysteinemia promotes oxidant damage to vascular cells by several mechanisms: auto-oxidation, elevated production of ROS in platelets and homocysteine thiolactone interaction.

Free radicals (FRs) and Reactive Oxygen Species (ROS), predominantly oxyradicals, e. g. superoxide ($O2^{-}$), hydroxyl ion ($OH^{\cdot}$), are continually produced during metabolic processes. As a result of the excessive generation of FRs, oxidative stress overwhelms the antioxidants available (redox imbalance) and stimulates some reactions causing cellular damage or cell death (4). Oxidative stress impresses on cell membrane polyunsaturated fatty acids -contained lipids and commences lipid peroxidation which is used as determinant of oxidative stress and cellular injury indicator **[5]**. One of the end products of lipid peroxidation with low-molecular-weight is MDA. It is the most abundant and reliable biomarker to measure the degree of lipid peroxidation and the levels of free radicals indirectly.

Acetylated derivative of neuraminic acid is sialic acid (SA) that is widely distributed in throughout vertebrate tissues, body fluids and in higher invertebrate species. **(Lacomba *et al*., 2010)**. It indwells the terminal location on macromolecules and cell membranes and participates in many biological and pathological phenomena. Since the majority of sialic acid is found ineither protein (PBSA) or lipid-bounded (LBSA) forms and usually is bound to glycoproteins, glycolipids, oligosaccharides and polysaccharides, paucity amount of it is in the free form. Moreover, it is involved in the end chain of many acute phase proteins and it is known as an inflammatory marker. **(Haq *et al*., 1993)**. Sialic acid is joined to non-reducing residues of the carbohydrate chains of glycoproteins and glycolipids. Glycosylation and sialylation of lipids and proteins carried out in the liver. Evidence shows that the changes in the sialylation of proteins and lipids have a substantial role in the pathogenesis and development of diverse liver diseases. **Chrostek *et al*., (2011)**. The H pylori-associated SA studies is scarce, for example, ameliorative effect of SA on eradication of *H pylori* infection has been assessed, **(Yang *et al*., 2013)** but SA alterations in serum has not been yet evaluated.

Hepcidin, as the 25-amino-acid peptide is mainly synthesized in the liver. It is the essential hormone in regulating iron homeostasis and Hepatic secretion of hepcidinis associated with response to iron overload. At the molecular level, Hepcidin mechanism in iron homeostasis is related toinhibition of iron efflux from enterocytes, macrophages and hepatocytes into the plasma by inducing internalization and degradation of the iron exporter ferroportin in these cells. **(Sam *et al*., 2013; Lee *et al*., 2010)**.

Sphingosine 1-phosphate (S1P) is one of the sphingosine derivatives which belongs to sphingolipids group. S1P is known as an intracellular mediator affecting survival and cellular proliferation **[1]**. Later studies showed that S1P is plentifully existed in plasma and other body fluids, where it acts in an autocrine or paracrine component to regulate important physiologicand pathophysiologic processes. **(Nofer, 2008)**. S1P plays crucial role as an extracellular mediator with a various cellular responses, including survival, proliferation, migration and contraction. So far, S1P has been revealed to be involved in theregulation of essential physiological functions of the vascular system, such as vascular morphogenesis and maturation, cardiac function, vascular permeability, and tumor angiogenesis **[13]**. In addition, S1P has been shown to be essential for lymphocyte egress from the secondary lymphoid tissues to the lymph.Very recently, S1P have been furtherrevealed to play an important role in bone homeostasis **[23]**. Thus, thesefindings forcefully suggest that S1P has important roles in vivoas well as potentially pathophysiological roles as a circulatingparacrine mediator **(Nofer, 2008)**.

Iron is an essential component of hemoglobin and myoglobin and of many enzymes involved in redox reactions and energy metabolism. Low levels of iron in the circulation may cause severe dysfunctions (e.g., anemia, hypoxia) while iron overload may be toxic because of its ability to generate reactive oxygenspecies. Excessive dietary iron uptakemay cause iron deposition inmany vital organs, including the liver, heart, skin, and especially pancreas **(Pietrangelo 2004, Beutler 2006; Kulaksiz *et al*., 2008)**. Iron displays important interactions with other essential microelements such as zinc and copper, showing competitive inhibitions in their transport and bioavailability. Copper is known to be as an major catalytic cofactor in a number of critical enzymes that are used in biological functions for growth and development **[13]**, **(Janjetic *et al*., 2010)** and zinc involves in multiple roles such as physical growth, immune competence, reproductive function and it is essential for the survival and function of cells. Decreased levels of serum zinc concentrations might be asscribed with gastric mucosa inflammation induced by *H.pylori* **(Akcam, 2010; Zhang *et al*., 2012)**.

To our knowledge, no assessment has been performed to investigate some clinical pathologic findingsin *H pylori* affected rat. Hence, this is the first work evaluate above-mentioned parameters.

MATERIALS AND METHODS

Preparation of *H pylori* suspension

For preparation of *H pylori* suspension, the fresh culture colonies were transferred to Muler-Hilton Broth and then its turbidity was regulated through 0.5 Macfarland tube (approximately 1.5×10^8 bacteria/ml).

Procedure of study

In this trial, 12 Albino male Wistar rats (175±15g , with average age one month) assigned into two groups in special cages under standard hygienic conditions and were allowed to use water and standard pellet *ad libitum* and 12:12 h light: dark at temperature 21-25^{oC} with 39% humidity. After 10 days of acclimatizing1ml of prepared suspension transferred via gavage to 6 rats (treatment group) and the same amount of distilled water transferred to control ones (6 rats). Three months later,all rats were anesthetized (by sodium pentobarbital, 50 mg/kg, i.p) and blood samples were taken by intra cardiac puncture and then transferred to heparinized tubes and centrifuged at 4000 RPM for 10 minutes at 4℃and prepared plasma were frozen in -25℃ until analysis.

Biochemistry parameters measurement

TSA concentration was determined through Sydow method. The ELISA kit (Bioassay Technology Laboratory Co, China) was utilized for plasma Hep concentration. Plasma S1P was detected by ELISA kit (East Biopharm Co, Hangzhou, China). Plasma MDA concentration was measurred by Satoh method and Cu, zinc, ADA along with Hcy were determined by Spekol 1500 spectrophotometric device (Parsazmoon Co kits, Tehran, Iran).

RESULTS

Alterations of all parameters have been denoted in **Table 1**. The results indicate significant increase (p<0.01) in all parameters in treatment group in comparison with the healthy ones.

Table 1: Alterations of some plasma parameters in treatment group compared with control ones

Parameters	Control Group	Patient Group
Hcy (nmol/l)	5.98 ± 1.35	11.14 ± 3.02†
ADA (U/L)	15.84 ± 4.22	42.64 ± 6.65†
TSA (mg/dl)	20.4± 5.83	39.95 ± 4.07†
S1P (ng/l)	95.27 ± 12.34	231.85 ± 46.28†
Hep (pg/ml)	67.23±4.12	262.52±36.91†
MDA (nmol/l)	2.36 ± 0.83	7.65 ± 0.79
Cu (µg/dl)	54.48 ± 8.7	149.51 ±12.45†
Zn (µg/dl)	69.23 ± 6.63	236.52 ± 16.29†

NOTE: Data are expressed as mean ± standard deviation. † Significantly different from the control group (P<0.01)

DISCUSSION

Total sialic acid (TSA) levels are increased in this study. Many studies have revealed that TSA elevation is occurred during pathologic conditions, proliferation and tissue inflammation. **(Nayak and Roberts, 2006)**. In Stefenelli study in 1985, sialic acid serum concentrations was higher than the control group. **(Stefenelli *et al*, 1985)**. In addition, TSA increase has been reported in other studies such as diabetes mellitus, myocardium infarction, tumor and alcoholism. **(Voigtmann *et al*, 1989; Po¨nnio¨ *et al*, 1999; Crook *et al*, 2001)**. Above mentioned studies is in accordance with our study. The probable reason of TSA increase can be due to cytokines secretion from defense cells and also it is seemed the SA may acts as a linkage for *H pylori* junction because the glycoprotein components have surrounded *H pylori* from stomach acid damage.

Plasma Hepcidin was high in treatment group than control ones. During infection, the Hepcidinincrease is induced for iron bioavailability decrease. **(Galesloot *et al*, 2011)**. The serum Hepcidin elevationhas been reported in pneumonia, sepsis and pyelonephritis and IL-6 causes Hep increase in diseases. **(Tomosugi *et al*, 2006)**. *H. pylori* ruins the iron regulatory mechanism that is useful to *H. pylori*, but deleterious to the host, by inducing the up-regulation of hepcidin and/or down-regulation of ferroportin **[14]**. **(Lee *et al*., 2010)**. Hence, it is possible that due to inhibitory effects of physiologic system, Hepcidin expression is elevated for decrease of *H. pylori* proliferation through decline of Iron bioavailability and/or *H. pylori* induces Hepcidin expression through unknown mechanisms.

The significant increase of S1P has been revealed in this study. **Li *et al*., 2009** demonstrated high activity of sphingosine 1 phosphate in plasma during the occurrence of gastric cancer and subsequently S1P is elevated which is in accordance with our study. We could not find any study which is related to S1P alterations in *H pylori* infection. The S1P increase may be attributed to releasing of S1P from platelet in gastric ulcer because platelet is known as one of the essential sources of S1P.

Our results in respect of Cu levels in plasma showed an increasein*H pylori*group than control ones which is not in accordance with **Toyonaga *et al*., [37]** study, who revealed a significant decrease inserum Cu concentrations between *H pylori*–positiveand –negative ones in Japan. It is possible that high level of Cu may be attributed to infection, because increase in serum copper concentration has been reported in inflammation and infectious diseases in animals and human **[38, 39]**. Significant increase of plasma zinc has been revealed in this study. The relation between *H pylori* infection and zinc has not beenextensively investigated. Our results are not in accordance to thosereported by **Akcam *et al* [40] and Janjetic *et al*.**, in children with *H pylori* infection. **(Janjetic *et al*., 2010)**. But it is in accordance with **Dovhanj *et al*** study who reported increase of plasma zinc and copperconcentrations in the *H. pylori* infection and this increase was not associated with tissue damage.

Elevated amounts of MDA have been determined in*H pylori*-infected rat rather than control group. Lipid peroxidation can damage proteins, lipids, carbohydrates and nucleic acids and plasma membranes are the critical targets of lipid peroxides **[26]**.

Navvabi *et al* and Vijayan *et al* revealed increased levels of MDA in H pylori infected ones which is in consistent with our study. Reactive oxygen species (ROS) generated by *H pylori*might be one of the essential factors whereby lipid peroxidation can act a role in the production of MDA **(Arend *et al*., 2005)**.

In respect of plasma Hcy, significant increase was showed in infected group rather than healthy ones. **Tamura *et al* in 2002** reported elevated levels of Hcy in *H pylori* infected group and related to low concentration of folic acid and vitamin B12. They refer mal-absorption of those vitamins in *H pylori* infected ones whereby trans-methylation is disturbed and finally Hcy can`t be converted to methionine. Our study is in consistent of above-mentioned study. In contrast, Rasool et al in 2012 revealed no relationship between H pylori infection and Hcy alterations in functional dyspepsia patients which is not in accordance of our study.

Namoit *et al* in 1990 showed increased activity of ADA in *H pylori* positive group. In this study plasma activity of ADA was high that is in agree with Namoit study. In conclusion, *H pylori* causes some plasma parameters alterations in rat and it is necessary more investigation for recognizing of biochemical biomarker among them.

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