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Research Article

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Assessing the effect of vitamin E oral administration on oxidative stress level of stomach tissue in diabetic patients infected with Helicobacter Pylori

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ABSTRACT

Introduction and Aims: *It is considered amplification of Immune system anti- oxidants cell in Diabetes Mellitus can be an influential factors in decreasing diabetic's effects and Helicobacter Pylori contamination, such as vitamin E has ante oxidants characteristics. SO this research aim is investigated the effect of edible prescription vitamin E on the improvement of stress oxidative' consequences results from H. Pylori contamination at stomach textures of diabetes mellitus.*
Martials and Method: *In this study 43 diseases H. Pylori contamination is choose as a sample, and are treated by controlling H. Pylori contamination .Second group is named the case group, in addition to treat by controlling H. Pylori contamination routinely they are received vitamin E . Measurements of superoxide dismutase and glutathione peroxide enzymes activity are done by UK RANDOX manual kit.*
Results: *At the case group activities rate of superoxide dismutase enzyme and glutathione peroxide are compared the sample group after edible prescription vitamin E, and statistically is shown significant increase. P value for activities rate of superoxide dismutase enzyme is less than 0.0001 and for glutathione peroxide equals 0.005 statistically was more significant.*
Conclusion: *The results of the study are shown that edible prescription vitamin E can improve capacity of antioxidant mocks textures. The improvement can help preventing the extension of diabetes mellitus complication and H. Pylori contamination and at the other hand increased the host strength against H. Pylori.*

Keywords: *H. Pylori, Diabetes Mellitus, Vitamin-E, Oxidative Stress*

INTRODUCTION

Diabetes mellitus is a complex metabolic syndrome which is caused by impaired glucose metabolism. Oxidative stress has been shown to play a role in the production of secondary complications of diabetes types 1 and 2 by measuring lipid peroxidation and protein factors (1 and 2). And increased blood glucose level increases the synthesis of free radicals, resulting in oxidative state. Additionally, overproduction of reactive oxygen species (ROS) or the removal of these compounds, which is mainly caused by hyperglycemia, leads to oxidative stress, exacerbation of atherosclerosis, capillary complications, degradation of fat, protein and DNA and LDL oxidation in diabetes (3 and 4). Unfortunately, the precise mechanism of oxidative stress in diabetes is unclear, although the available evidence indicates the association between the increased reactive oxygen species, such as superoxide and hydrogen peroxide

and reduced antioxidant defense (5 and 6). This mechanism includes auto-oxidation of glucose, the activation of polyol path and important enzyme responsible for ROS and nitrogen from the corresponding precursor, such as molybdenum hydroxylases enzyme, i.e. xanthine oxidoreductase which is effective on Hypoxanthine and xanthine, the activation of aldehyde oxidase effective on purines, the inactivation of antioxidant enzyme, such as superoxide dismutase catalase, glutamine peroxidase, a significant reduction in non-enzymatic antioxidants, such as vitamin E and vitamin C, the disorders in prostaglandins metabolism and nitric oxide (NO), and insulin resistance (7 and 8). A proper process is to prevent the production of reactive species or to neutralize them, so antioxidants (especially anti-natural kinds) are considered suitable candidates for the prevention of oxidative stress in diabetic complications (9). Vitamin E is one of the nutrient compounds which besides having a high antioxidant feature, has effects on various biological processes in the body, including immunity (10), metabolism (11) and reproduction (12). In recent years, diabetes mellitus has also been addressed as the main cause of irregularities in the gastrointestinal-intestinal tract (13). Due to the fact that *Helicobacter Pylori* also cause many cases of dyspepsia, the increased prevalence of *H. Pylori* infection have been reported in individuals with diabetes (14). The delayed gastric emptying and reduced gastric antral part are the complications yielded by dyspepsia in diabetes. The role of *H. Pylori* infection in diabetic dyspepsia is originally attributed to hyperglycemia. Hyperglycemia may induce the *H. Pylori* infection, or activate a mild or inactive infection without any signs and symptoms, and may lead to the indigestion in diabetes. *H. Pylori* infection in diabetic patients who have not been metabolically controlled is common and is colonized in the gastric antrum of these people (15). Although much has been carried out on *H. Pylori* and that the bacterium has received considerable attention throughout the world, there still remain large gaps in our knowledge of understanding the transmission mechanism, colonization, isolation, cultivation and eradication of the bacteria. This pathogen can successfully stay in the stomach tissue of an infected person and cause serious disruption, such as severe stomach ulcers, gastric cancer and gastric lymph nodes adenocarcinoma, to the life of infected persons (16 and 17). If the bacteria can be completely eradicated from the stomach tissue, these serious complications can be prevented, too (18). Currently, there are several types of antibiotics to treat the infection, but it is unfortunate that the drug treatment has not been very effective, and is accompanied by very serious side effects, such as losses of hearing and pharyngitis as well as *H. Pylori* resistance to these antibiotics occurs in most cases (19 and 20). A very high risk of infection return, the high cost of antibiotic, and the lack of strong and effective vaccine against this infection have drawn the researchers' attention to think about finding new therapies to rule out this global infection (21).

On the other hand, superoxide radical bacillus form changes into coccoid form over the *H. Pylori* bacteria transformation. As a result of the reaction between superoxide radical and nitric oxide, two metabolites are produced that are extremely toxic to different organisms and cause oxidative stress reactions in the interested tissue (22 and 23). The high concentration of superoxide radicals produced by *H. Pylori* may increase superoxide dismutase activity in gastric mucosa. SOD is counted as a key enzyme for the removal of various types of reactive oxygen radicals, such as superoxide radical, to protect tissues against oxidative damage and to maintain tissue homeostasis (22 and 24). Therefore, evaluating the activity of superoxide dismutase is regarded as a significant factor in studying oxidative lesions of *H. Pylori* oxidative infection and diabetes mellitus. According to the studies conducted on the patients who were infected with *H. Pylori*, the mean activity of gastric mucosa superoxide dismutase (superoxide dismutase in gastric mucosa) was much higher than that in the individuals uninfected with *H. Pylori* (25). In examining the damages caused by *H. Pylori* infection and lesions of oxidative stress in diabetes mellitus, determining glutathione peroxidase activity is also the case. On the basis of the studies on gastric mucosal lesions and oxidative stress, depletion of glutathione and alpha-tocopherol from gastric mucosa, has been reported to induce the production of free radicals (26 and 27). As a result, this can serve as a secondary cause in the development of oxidative lesions. Therefore, to carry out a proper and further evaluation of lesions, identifying the activity of glutathione peroxidase enzyme in the gastric mucosa can be helpful as a supplement parameter.

Therefore, in line with the studies, the aim of this study was to investigate the effect of oral supplementation of Vitamin E on improving oxidative stress complication induced by Helicobacter pylori infection in the stomach tissue of diabetics.

Materials and methods:

This study was a double blind clinical trial intervention whose target population consisted of diabetic patients infected with Hp.

Selection and sampling the participants:

Diabetic patients, who had gastrointestinal symptoms and referred to the gastrointestinal clinics in Tabriz University of Medical Sciences, were recognized by a gastrointestinal specialist and referred to the Endoscopy Unit of Imam Reza Hospital for endoscopy. First, each of the subjects were examined in terms of other diseases (e.g. gastric cancer, kidney disorders), smoking, taking antioxidants drug, antacids, bismuth and other cases which would cause false effects in our study, and if so, they were excluded from the study. Then, the endoscopy was performed by a gastroenterologist on the selected individuals. Respectively, the patients were randomly divided into two groups: a group of diabetic patients infected with *H. Pylori* were selected as the control group, received the conventional therapy of *H. Pylori* infection; and another group of diabetic patients infected with *H. Pylori* as the case group, received the conventional treatment of *H. Pylori* infection accompanied by vitamin E, 15 mg per day for 3 weeks. Of the patients, three biopsy specimens were taken from the gastric antrum within 3 centimeters of the pylorus and a whole blood sample in vials containing anticoagulant substance and another whole blood sample for serum measurements in fasting conditions. Two samples of these biopsies were used for rapid urease test and for direct smear. It should be noted that by providing the direct smear of biopsy specimens and staining it with the Gram method and examining the presence or absence of *H. Pylori*, the urease test results that might have false results, were confirmed. In this way, the biopsies were homogenized by a homogenizer, and then the direct smears were prepared by them, and stained by Giemsa method. The third biopsy sample was used to measure the activity of superoxide dismutase and glutathione peroxidase enzymes. The whole blood sample containing anticoagulant blood agent was used to measure glycosylated hemoglobin percentage, and the whole blood sample without anticoagulant agent was applied for fasting blood glucose serum (FBS) measurement. It should be noted that the obtained samples were stored at -70° C in a freezer until the time of testing.

In addition, before obtaining the samples, the patients were asked to give personal information about their ages, gender, history of their other diseases, and then it was recorded in the checklist prepared for this purpose, and both groups were homogenized.

Superoxide dismutase and glutathione peroxidase enzymes activity measurement was performed by a manual kit and colorimetric manufactured by RANDOX (RANSOD- Superoxide dismutase MANUAL).

The activity of these enzymes should have been calculated on the basis of gastric mucus protein level. So, gastric mucus protein was also measured. Since the level of protein in gastric mucosa is variable, so a high sensitivity method was required to determine the amounts of protein in gastric biopsies. Therefore, Laurie's method which is the combination of the two Biuret and Folin-Ciocalteau methods was recommended by all the researchers who have been working in this field.

Data analysis (statistical methods):

First, because of the independence of the study groups, the mean of the results was calculated by SPSS statistical software for each group. And normal distribution of results was evaluated by Shapiro Wilks test which were normally distributed, so the results were compared by using Paired Sample t-Test in the two groups. The tests were considered significant when the p value was less than 0.05

Results:

Comparing demographic data in the two study groups:

Demographic information of the two groups is shown in Table 1 which were statistically compared and, as can be seen, the study groups were homogenized in terms of age, blood pressure, fasting blood glucose, urea, cholesterol, triglycerides and glycosylated hemoglobin (in all cases $p > 0.05$).

Table 1: Demographic data of the patients in the two study groups

Groups Clinical factors	Control Group (N = 32) Mean ± SD	Case Group (N = 32) Mean ± SD	P value
Age (years)	60.94 ± 14.44	63.43 ± 15.53	0.499
Systolic blood pressure (mmHg)	112.65 ± 16.16	116.87 ± 17.21	0.604
Diastolic blood pressure (mmHg)	73.28 ± 11.88	76.87 ± 10.68	0.588
Fasting blood glucose (mg/ml)	150.12 ± 30.15	154.92 ± 36.02	0.660
Urea (mg/dl)	58.96 ± 36.57	61.56 ± 43.58	0.270
Cholesterol (mg/ml)	185.73 ± 39.34	115.45 ± 36.53	0.896
Serum triglyceride (mg/ml)	140.27 ± 29.69	119.32 ± 31.42	0.873
Glycosylated hemoglobin (percent)	11.19 ± 2.49	11.06 ± 2.14	0.429

Comparing the average level of gastric mucosa tissue antioxidant enzyme activity in the two study groups:

As can be seen in Table 2 and Figures 1 and 2, by using SPSS version 21 and Independent Samples t-Test test, it turned out that after oral administration of vitamin E, superoxide dismutase and glutathione peroxidase enzymes activity was statistically significant and showed an increase in the treatment group in comparison with that of the control group. Results are shown as mean ± SD which showed to be 13.03 ± 1.17 and 15.9 ± 3.38 (IU/ mg Protein) for superoxide dismutase enzyme activity after treatment in both groups, respectively. And p-values for this comparison were less than 0.0001, which was highly significant. In addition, the mean ± SD of glutathione peroxidase activity after treatment in both groups was respectively 6.37 ± 1.66 and 8.72 ± 3.11 (IU/ mg Protein), and p-values for this comparison was equal to 0.005, which was statistically highly significant.

Table 2: Data on comparing the average level of antioxidant enzymes activity in the two groups before and after treatment

Groups Enzyme Activity	Control group (N = 32) Mean ± SD	Treatment group (N = 32) Mean ± SD	P-value
Superoxide dismutase (IU/ mg Protein) Before treatment	4.39 ± 2.29	4.28 ± 2.03	0.339
Superoxide dismutase (IU/ mg Protein) After treatment	13.3 ± 1.71	15.90 ± 4.38	<0.0001
Glutathione peroxidase (IU/ mg Protein) Before treatment	1.36 ± 0.92	1.43 ± 1.08	0.442
Glutathione peroxidase (IU/ mg Protein) After treatment	6.37 ± 1.66	8.72 ± 3.11	0.005

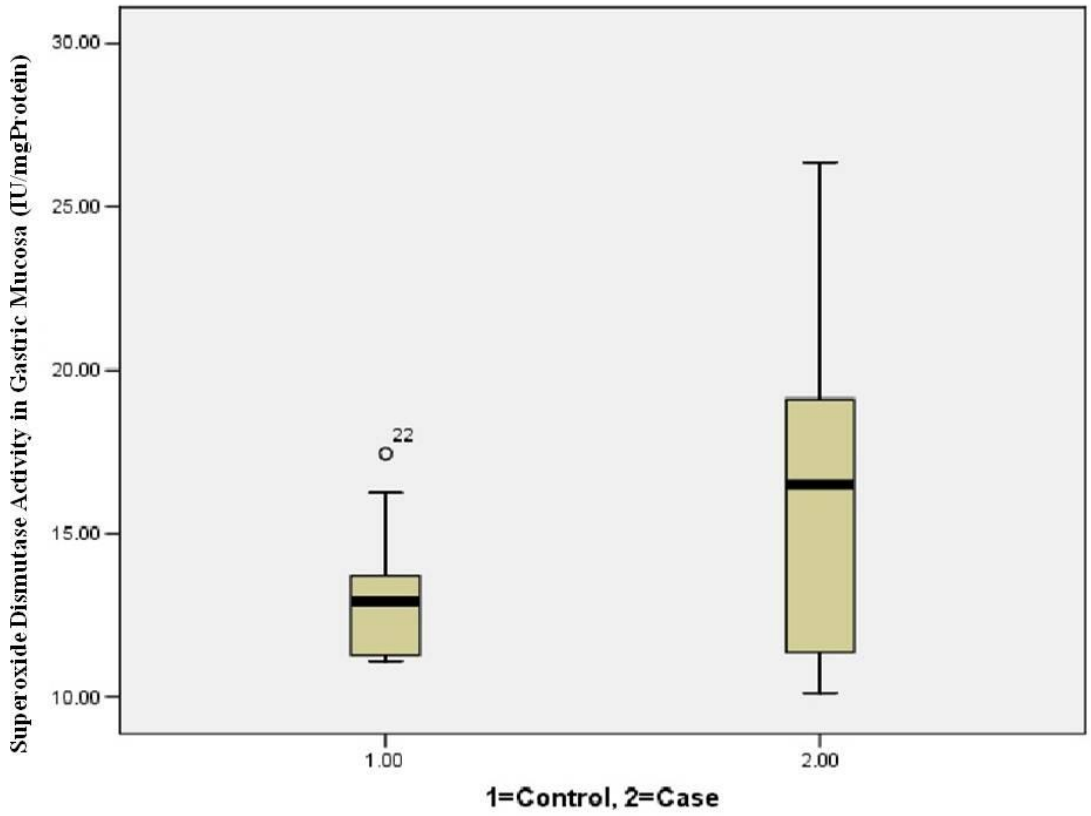

Figure 1: The curve of the comparison of superoxide dismutase enzyme mean activity after treatment in the two groups.

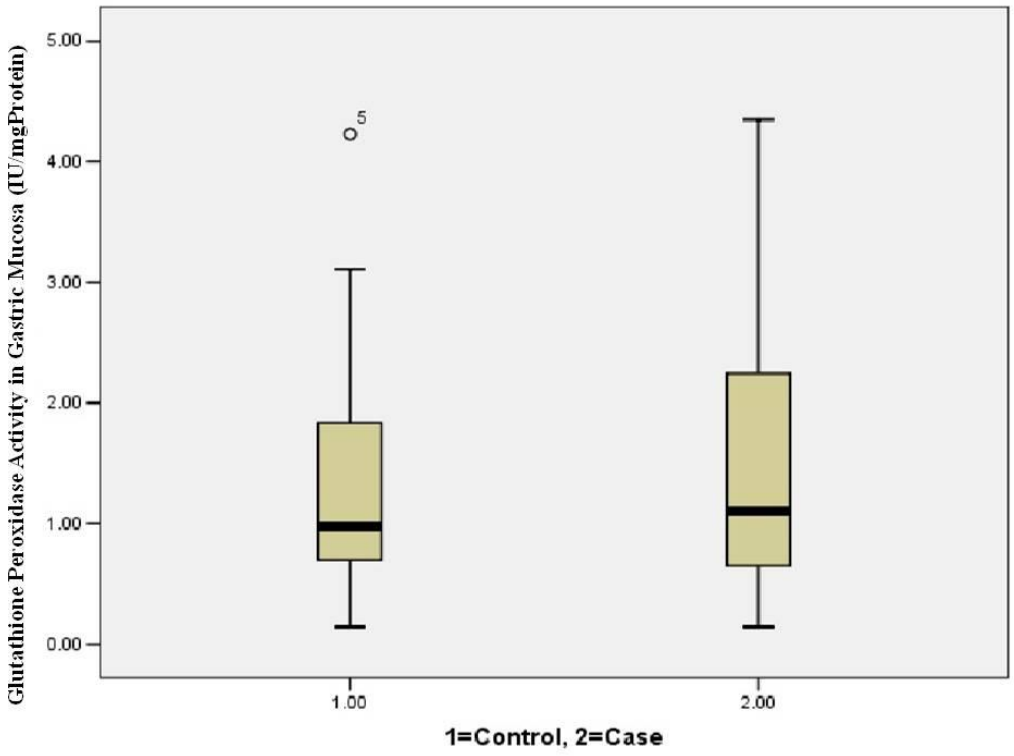

Figure 2: The curve of the comparison of glutathione peroxidase mean activity after treatment in the two groups.

Discussion:

Infection with *H. Pylori* and diabetes mellitus are of major concerns and priorities of general Hygiene to modern and developing societies, as the both cause the risk of cardiovascular disease (4), cancer (5) and an increase in Metabolic abnormalities (6). Moreover, in recent years, diabetes mellitus is considered as the main cause of irregularities in gastrointestinal tract activities (1). Due to the fact that *H. Pylori* also causes many cases dyspepsia, the increased prevalence of *H. Pylori* infection has been reported in diabetic patients (2). Delayed gastric emptying and decreased gastric antral function are regarded as indigestion complications in diabetes. The role of *H. Pylori* infection in diabetic dyspepsia is originally attributed to hyperglycemia. Hyperglycemia may irritate the *H. Pylori* infection or may reactivate a mild and inactive infection which has been without any signs, and may manifest indigestion symptoms in diabetes. *H. Pylori* infection in diabetic patients, who have not been controlled in term of metabolic, is common infection, and in these people, *H. Pylori* is colonized in the gastric antrum (3).

Some studies have rejected the association between *H. Pylori* infection and not supported the relationship between diabetes metabolic control and *H. Pylori* infection (7). While other studies revealed *H. Pylori* infection and glycemic control in patients with diabetes in comparison with those of in nondiabetic patients. Furthermore, the relationship *H. Pylori*, insulin and serum glucose level has been shown (1 and 8-11).

According to the conducted studies, it turned out that glycemic control in diabetic patients may be useful when *H. Pylori* infection is eradicated possibly by decreasing the level of glycosylated hemoglobin to almost the same level as with that of the uninfected group with *H. Pylori* (6). In other words, it was assumed that the individuals with diabetes and infected with helicobacter, who had a higher percentage of glycosylated hemoglobin than the individuals uninfected with *H. Pylori* showed resistance to drug therapy, may increase the level of gastric tissue antioxidant enzymes, i.e. superoxide dismutase and glutathione peroxidase enzymes with the use of vitamin E as an oral supplement and a powerful antioxidant. This may treat secretion disorders and NO^{o} production. Additionally, it may alleviate the symptoms and lesions caused by helicobacter pylori infection, and oxidative stress resulted from metabolites of the reaction between nitric oxide and superoxide radical. Several studies published in recent years have made known that *H. Pylori* strains isolated from gastric mucosa have phenotypic and genotypic changes that may cause various inflammatory reactions, and thus it may have an impact on clinical outcomes (28). Although the results of the further investigation have identified most of *H. Pylori* mechanisms and virulence factors, clinical and laboratory findings are controversial yet in this regard, requiring undergoing further research.

In this study it was manifested that the activity of superoxide dismutase and glutathione peroxidase enzymes in the gastric mucosa was increased because of oral administration of vitamin E along with therapy with antibiotics to eradicate *H. Pylori* in *H. Pylori* infected individuals. The following discussion focuses on this issue.

Several studies in recent years have provided compelling evidence about the possible role of oxidative stress in the development of diabetic complications. However, in some studies, detailed clinical assessment has rejected the possible role of antioxidants, especially vitamin E to show any effectiveness. But recently, it was suggested that antioxidant therapy with vitamin E or other antioxidants was considered more as a symptomatic treatment for oxidative stress.

One of the difficulties arisen in connection with *H. Pylori* infection in stomach tissue is the incidence of oxidative stress (13 and 14). Oxidative stress is defined as a condition in which the cells are exposed to high levels of oxygen molecule or its chemical derivatives of the so-called Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (15). Strictly speaking, oxidative stress is created as a result of an imbalance between the production of free radicals and reactive oxygen species on the one hand, and the antioxidant defense system on the other hand. In aerobic biological systems, in order to cope with free radicals and reactive oxygen species, antioxidant defense mechanisms are designed to neutralize the damaging effects of these striker factors or to minimize them. Some components of this defense system, such as superoxide dismutase, glutamine peroxidase, catalase enzymes as well as uric acid, bilirubin and thiol molecules are produced within the body, while others, such as vitamins E or alpha tocopherol, vitamin C and beta-carotene must be supplied through diet. Many of the macromolecules are injured in oxidative stress.

Consequently, lipid peroxidation process, DNA oxidation, protein oxidation, enzyme inactivation and dysfunction of various membranes occur. The available evidence supports that oxidative stress is involved in the pathogenesis of more than one hundred diseases (16).

Of the absolutely essential antioxidants is vitamin E. Vitamin E consists of a group of compounds that include both alpha-tocopherols and tocotrienols with different biological activities. Alpha-tocopherol as the most frequent gastrointestinal form of vitamin E is one of the most important fat-soluble antioxidants, which could easily pass through biological membranes. According to the results of a study conducted by Syse et al. 1995 (29), it was found that this vitamin is able to, by increasing the activity of natural killer cells, play a very important role in increasing human immunological response. Besides, researchers showed that in patients with *H. Pylori* infection, with increasing concentrations of mucosal Vitamin E, a smaller number of bacteria exist in the gastric mucosa and duodenal cofactor. Pauli et al. (1996) (30) presumed that this phenomenon probably indicates the antioxidant defense mobilization into the sites of inflammation in stomach. In addition, Zhang et al. (2000) (31), suggested that the intestinal metaplasia and gastric atrophy are significantly reduced by increasing alpha-tocopherol concentration in the tissues of stomach. They also reported that in individuals whose gastric antrum is infected with *H. Pylori*, mucosal concentrations of alpha-Tocopherol is reduced in the gastric antrum. As a result, gastric antrum gradually shows mucosal tissue changes which may be chronic gastritis or atrophy and intestinal metaplasia.

However, a number of studies have expressed beneficial effects of vitamin E on reducing complications of diabetes mellitus, the results of which are fairly consistent with the results of the current one. For example, Park et al. (2002) (32) reported that in diabetic patients who were continuously injected with insulin, the effect of vitamin E on lipid peroxidation has been very useful. Manning et al (2004) (33) described that taking vitamin E orally by diabetics can improve complications caused by oxidative stress, can improve liver function in these patients, and may also reduce insulin resistance in them, the result of which can approve the current results. By measuring the amount of glucose, lipid profile, apoprotein B and glycated hemoglobin, Paolisso et al. (1993) (34) published that oral administration of vitamin E daily to type 2 diabetic patients can be effective in metabolic control in these patients, but they noted that further studies need to be carried out in line with the results of this study. Kahlr et al. (1993) (35) reported that in diabetes mellitus, free radicals and oxidative stress play crucial roles in the development of diabetes complications, and the use of antioxidants, especially vitamin E prevent these complications, which would be fully consistent with the current assumptions. On the other hand, a study by Beydoun et al. (2015) (36) conducted on diabetic patients infected with *H. Pylori*, shed light on two completely direct path through which *H. Pylori* infection in diabetics decreases significantly the antioxidant whole capacity and reduces the amount of iron. These researchers suggested that in a randomized clinical trial with a control group and with the use of antioxidant supplements, after eradication of *H. Pylori*, the amount of iron, folic acid, vitamin B-12 and antioxidant concentration were measured and then, the patients' improvement was assessed. In line with it, this study partly accomplished the proposal of Beydoun et al (36).

In acute and chronic gastritis caused by *H. Pylori*, neutrophils form the initial inflammatory response to pathogen. In activated neutrophils, the NADPH oxidase enzyme is activated, and the transfer of one electron from NADPH to oxygen occurs in and out of cells, and oxygen molecules receiving electrons are converted to superoxide radical ($O2^{-}$) which are rapidly transformed to hydrogen peroxide (H2O2) and hydroxyl radicals by superoxide dismutase (ºOH). Production of reactive oxygen species (ROS) by the host cells and bacteria in biopsy specimens of duodenal and of stomach of patients with *H. Pylori* has been displayed in a vast number of investigations (37). And it has been published that the source of reactive oxygen species (ROS) production is host neutrophils which are activated by soluble factors of *H. Pylori* (38) as well as being secreted by the bacteria itself (39). In their project, Suzuki et al (2012) (40) suggested that the production of reactive oxygen (ROS) in the mucosa of the stomach infected with *H. Pylori* goes up with accumulation of neutrophils in patients with peptic ulcer. SOD catalyzes superoxide anions dismutasion ($O2^{-}$) to hydrogen peroxide and molecular oxygen (41 and 42). Though, H2O2 may be increased as a result of its excess release from the neutrophils activated by soluble factors of *H. Pylori* (43).

Glutathione (GSH) is regarded as the main non-protein thiol in living cells and endogenous antioxidant that is found in high concentrations in the stomach and liver. GSH concentration is higher in gastric tissue than in other parts of the gastrointestinal tract and other organs (44 and 45). GSH in cells is largely reduced and act as a collector of free radical and also facilitates the reproduction of other antioxidants such as vitamin E. Cleansing and detoxification of hydrogen

peroxide (H2O2) and lipid Hyperoxides (LOOH) produced by glutathione peroxidase (GPX) in *H. Pylori* infection can be started by using GSH (45). Some studies have shown that GPX are located in gastric mucosa in epithelial and parietal cells, and this enzyme is also responsible for redox regulation of their activities. Reports on the effects of *H. Pylori* in the content of GSH and GPX enzyme in the gastric mucosa are contradictory. This is while, the results of other researches state that after colonization of helicobacter pylori, GSH of gastric mucosa may be reduced due to the direct effect of the bacteria or may be enhanced due to its function as an antioxidant defense and actually as a substrate for GPX activity (46 and 47). It has recently been proposed that it is possible that, due to poor absorption of selenium or its redistribution of plasma supply inside body tissues, GPX activity is lessened, because selenium is an essential element for GPX catalytic activity (46). The decreased GPX may also account for its reduced synthesis in the kidneys, because it is believed that the main source of the enzyme activity and production lies in the cells of the proximal renal tubules (47). However, it is expected that in antioxidant defense system, GPX function would be SOD activity complementary and there must be a relationship between the two enzymes.

Conclusion:

In the present study, we showed a significant increase in antioxidant superoxide dismutase and glutathione peroxidase after the intake of vitamin E by the patients infected with *H. Pylori*. Therefore, we concluded that the antioxidant capacity of gastric mucosa can be improved. This improvement may prevent diabetes and *H. Pylori* infection and increase the power of the host against *H. Pylori*.

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