**Helicobacter pylori, vitamin-C & salt; A risk assessment of gastric cancer**

**Abstract**
Gastric cancer is still the forth most common cancer in the world. Many studies have showed that infection with *Helicobacter Pylori* is present in nearly all cases of gastric cancer but that the infection alone is not sufficient for development adenocarcinoma. Environmental factors and in particular diet have been suggested to be a contributor to the development of gastric cancer. Salt have been proposed to enhance the effect of *H.pylori* infection and also damage the epithelial cells of the stomach. The accumulation of an ulcer makes the inside of the stomach more sensitive to food carcinogens. The inflammation caused by continuous high salt intake seems to promote *H.pylori* colonisation and this further increase the inflammation and can develop into chronic gastritis. Salt have also been shown to increase the formation of endogenous N-nitroso compounds (ENOC), some of which are believed to be carcinogenic. Furthermore, Vitamin C has been shown in several studies to be inverse associated with gastric cancer. The mode of action is in terms of preventing gastric cancer is not known. As an antioxidant vitamin C functions as a scavenger. It is also known to block formation of carcinogenic N-nitrosocompounds. It also has potential to affect the immune response to infection and is an important co-factor for many enzymes. Studies *in vitro* has shown that vitamin C also functions as an anti-proliferative and pro-apoptotic compound. In this article presents epidemiological and experimental results to identify the potential role of vitamin C, its effect on *H.pylori* in gastric cancer. All studies examined support the protective effect of vitamin C. The results from the experimental studies viewed suggest that vitamin C has a direct effect on both cancer cells and *H.pylori*. 
1. Introduction
In the world today, cancer is increasing faster than the increase in global population. Predictions for 2030 estimate that today’s figures of 11 million diagnosed cancer cases each year will be doubled by this year[1].
Gastric cancer [GC] is the forth most common type of cancer in the world, it is the second most common cause of death from cancer [1,2]. However, the incidence of GC is decreasing in high-income countries it is still high in low-income countries [1].
Infection with the gram-negative bacteria Helicobacter pylori is present in nearly all GC subjects /1/. However, by estimation only 2,9% of the H.pylori infected develop GC [2,5,10], suggesting other complex interactions with the environment of the host to develop GC.
Salt and salty foods have been proposed to enhance the risk of GC through a few possible mechanisms which will be described in detail later [1,2,3,4,5,6,8,9,10]. Salt has been shown to damage the epithelial cells of the stomach, alter the mucus environment, increase endogenous N-nitroso compound formation and facilitate H.pylori infection [1,8].
The typical consumer consumes most of the salt from processed foods and not salt added in cooking or at the table [1]. This cause a problem when estimating total salt intake due to great variations in salt content of different foods. In some parts of Asia and particular in Japan, diets are traditionally high in salt content and the GC incidence is also very high in these countries [1,9,10]. The decrease in GC incidence seen in high-income countries from 1900 to 2000 is explained partly by the use of refrigeration as opposed to salt-preserved foods [1,2]. This is consistent with the observation that GC is more common in the older generation and low-income countries where refrigeration is or has been a rarity [1].
Vitamin C on the other hand has been shown to be protective against GC and is believed to several important roles in terms of preventing gastric cancer. It functions as an antioxidant and can inhibit the action of reactive oxygen species in the gastric environment. Furthermore vitamin C is known to inhibit N-nitroso compounds in the stomach. N-nitroso compound is known to be carcinogenic.[13]
Infection of H.pylori in the gastric mucosa is associated with increased infiltration of different inflammatory cells such as lymphocytes and neutrophils. Vitamin C may play several important roles in the effect of the immune response. By increase of T cell proliferation vitamin C stimulates the immune system in response to infection. Vitamin C may also protect neutrophils from oxidative damage.Furthermore, vitamin C is an important co-factor of many enzymes and is also thought to act as an anti-proliferative an and pro-apoptotic compound in vitro.[14]
This report will try to elucidate the potential risk factors H.pylori, salt and the potential preventive effect of vitamin-C.
2. Results

2.1. A brief overview of gastric cancer

GC exists in different forms: Distal GC, which is the most common form and cancer of the gastric cardia [or the gastro-oesophageal junction]. The distal GC can be further divided into intestinal and diffuse type. The former being the most common and is thought to be highly preventable [1]. The diffuse type show some genetic predisposition and people with blood group A seem to have an increased risk of this type of GC [1]. This type can also occur in the gastric cardia. However, this report will focus mainly on non-cardia GC and more specific on the distal/intestinal type of GC. The two major types of GC are histologically different. All distal cancers originate from the mucus producing cells and are thus adenocarcinomas.

2.2. Helicobacter pylori and gastric cancer

Research has found that 50-90% of the world’s population is infected with H. pylori [with higher percentages in developing countries], which make it the most common infection in the world [12]. Subjects infected can be without symptoms or with symptoms [5]. H. pylori colonises the gastric mucosa which results in chronic inflammatory and immune responses [1]. These changes result in gastritis and can with time develop into the more severe atrophic gastritis and intestinal metaplasia which are known precursors of GC [5]. Furthermore, subjects with H. pylori related pangastritis or corpus-predominant gastritis is more likely to develop intestinal metaplasia, gastric atrophy and hypochlorhydria [5]. Hypochlorhydria promotes the overgrowth of certain anaerobic bacteria which can convert dietary nitrate and nitrite into carcinogenic nitroso compounds [5].

Studies have shown that different H. pylori strains have a variety of virulence factors, these include: Cytotoxin-associated gene A [cagA], vacuolating toxin A [vacA], babA, opiA, sabA, alpA and alpB [7, 11]. These function as adhesins, invasins or toxins. The product of the cagA gene is the protein cagA which influence a number of factors in the host cell: cell proliferation, apoptosis [induces apoptosis in T-cells], morphological changes and induction of proinflammatory cytokines in epithelial cells [5, 11]. Moreover, cagA has been shown to [indirect] decrease p53 phosphorylation and increase Bcl2 expression [7]. These factors indeed have an impact on the severity of the inflammation and cellular function. Several studies have found that the vacA induces vacuolation on epithelial cells [such as parietal cells] in the stomach and causes cell death and reduced acid production. This leads to a more favourable environment for H. pylori colonisation and increase the risk of GC [5, 11]. VacA also seems to reduce the proliferation of T-cells which leads to increased risk of GC [11].

BabA, opiA, sabA, alpA and alpB are all adhesins. Limited amount of studies have been conducted on these molecules and the exact role is yet to be determined. Low levels of babA seemed to promote more severe mucosal injury, while opiA positive expression was associated with duodenal ulcer and gastric cancer. SabA was associated with GC and alpA, alpB was associated with low levels of proinflammatory effectors [7, 11].

Some genetic factors of the host also seem to influence the pathogenesis of GC [5]. Family history of the disease, functional polymorphism of the IL-1 and TNF-α genes have been associated with an increased risk of non-cardia GC [5]. This is true with subjects positive for H. pylori infection only [5]. Interactions between these host genetic factors and the virulence factors of the bacterium is proposed to contribute to the mucosal damage and physiological response associated with the increased risk of favourable environment for adenocarcinomas [5].
2.3. The effect of salt and salty foods on gastric environment

Salt and salted foods are considered a probable risk factor of gastric cancer [1,2,5,8,9,10]. Salt alone has been shown to damage the stomach lining [in animal studies], increase the endogenous N-nitroso compound formation [ENOC], enhance the action of carcinogens and may facilitate *H. pylori* infection [1,4]. However, most of the results are pointing towards an increased risk of GC with increased salt intake [1,4,5,8,9,10] some studies show no association or even a statistically significant decreased risk in one cohort study [1]. 12 case-control showed increased risk with increased intake [half of which were statistically significant] [1]. Of 7 ecological studies 4 showed statistically significant increase of GC with increased salt intake. Moreover, 5 other studies resulted in a non-significant decrease or no association [1]. Salt alone has *not* been proven to contribute to GC, only in presence of *H. pylori* infection and exposure to a chemical carcinogen [such as PAHs and other food carcinogens] [1,2]. Furthermore, colonisation of the bacterium result in a reduction in nitrate and increased formation of N-nitroso compounds [9].

In more detail, a high salt concentration in the stomach destroys the mucosal barrier which leads to inflammation, diffuse erosion and degeneration [3]. This enables other food carcinogens to enhance their effect in the stomach [3]. Furthermore, the damage caused by salt on the mucosal barrier is likely to promote a favourable environment for *H. pylori* to adhere to the inside of the stomach [3].

*H. pylori* moves with chemotactic motility towards the gastric mucus layer. To be able to resist the highly acidic environment of the stomach *H. pylori* has urease enzymes which converts urea present in the stomach into ammonia and carbamate [and further into another ammonia molecule and carbon-dioxide spontaneously] [11]. The ammonia increases the pH in the stomach.

Researchers have found that the gastric mucus consists of two different mucins: surface mucous cell mucin [SMCM] and gland mucous cell mucin [GMCM] [8]. *H. pylori* has not been found in the GMCM but in SMCM which is the dominant type in chronic gastritis [8]. During infection of the bacterium synthesis of GMCM is upgraded by unknown mechanism at transcriptional level [probably as a defence mechanism]. Salt, on the other hand seems to upregulate SMCM [which *H. pylori* prefers] and at the same time downregulates GMCM [11]. The role of salt in GC development can be illustrated by looking at past data. Over the last 50 years GC has decreased [age-adjusted incidence] without any attempt by society to eradicate *H. pylori* infected subjects [4]. Researchers have suggested that the major component altered during this period is the use of refrigeration instead of salt preservation of food. Which could point to some extent that salt is a major contributor to GC development in humans [4].

Of note is, that it is possible that the increased risk of GC by salt in fact could be attributed to possible carcinogens that form during the preservation process [6]. The fact that GC incidence is low in USA although salt intake is high needs to be considered [6].
2.4. Vitamin C and H. pylori

2.4.1. Epidemiological results
In the WCF/AICR report the authors drew the conclusion that vitamin C intake probably decreases the risk of gastric cancer. This report included 2 cohort studies and 13 case-control studies.

2.4.2. Prospective studies
One prospective study on Finnish male smokers were conducted with a 12 years follow-up. The authors examined the association between alpha-tocopherol, beta-carotene, ascorbic acid and one of the two subtypes of GC – gastric noncardia and gastric. The results from this study showed that vitamin C had a protective effect on specifically gastric noncardia cancer.[15]

A study by Wei-cheng et al. made a follow-up aiming to find risk factors for GC in high-risk populations. At study entry in 1989-1990 data on smoking, alcohol consumption was collected. Furthermore endoscopy was taken and antibodies to H. pylori assayed among 77% of the subjects. In 1994 the follow-up was made. Endoscopic examinations among the subjects were performed. Data was compared between subjects with signs of dysplasia or GC progression and subjects with no change or with regression of their lesions. The results showed that presence of H. pylori at baseline was associated with an enhanced risk of progression to dysplasia or gastric cancer. This risk was further increased with the number of years of smoking. Conversely, the risk was decreased with 80% among subjects in the highest tertile of baseline ascorbic acid levels compared to the subjects in the lowest.[16]

Furthermore, the levels of dietary and prediagnostic plasma vitamin C were compared between 215 GC cases and 416 controls in a nested case-control study in the European Prospective Investigation inte Cancer and Nutrition (EPIC) study. The results showed no association with dietary vitamin C and gastric cancer. But there was an inverse association observed in the highest versus lowest quartile of plasma vitamin C. This association was also stronger among subjects consuming high levels of red and processed meat.[14]

In another nested case-control study, a cohort of 20 000 Chinese men there was a significant inverse association with increased levels of serum of vitamin C and risk of GC among never-smoker and non-heavy-alcohol drinking men.[17]

2.4.3. Case-control studies
In a hospital-based case-control study including 295 cases of GC effects of dietary vitamin C on the relation between GC and H. Pylori were studied. Dietary vitamin C intakes were estimated from food frequency questionnaires and food composition tables. Furthermore, IgG antibodies against H. Pylori was measured. The results showed that there was a significant association between H. Pylori and gastric cancer. However, this association was significant only among subject with low vitamin C intake. [18]
2.4.4. Experimental studies

In an experimental study Zhang ZW et al examined the in vitro effects of vitamin C on GC cells and *H. Pylori* in vitro. They observed that vitamin C inhibited the growth of GC cells in a dose dependant manner and both DNA and protein synthesis were inhibited. This effect, however, was significantly reduced when concentration similar to those seen in the gastric juice of *H. pylori* infected patients. In the same study the effect of vitamin C in *H. pylori* associated cell cycle events was evaluated in GC cells. Further enhanced *H. pylori* associated apoptosis was observed and also cell cycle arrest was induced. Zhang ZW also examined the effect of vitamin C on the adherence ability of *H. pylori* to gastric epithelial but no effect was observed.[19]

Direct effects of vitamin C on *H. pylori* has also been examined in several studies. Jarosz et al examined the effects of high dose vitamin C administration and *H. pylori* infection and also the effect on vitamin C levels in the gastric juice in patients with *H. pylori* associated chronic gastritis. The results showed an eradication of *H. pylori* among 30% of the treated subjects (n = 27). The subjects recieved 5g of vitamin C per day for 4 weeks.[20]

Zhang HM et al examined the direct effects of vitamin C on *H. pylori* both in vitro and in vivo. The in vitro experiment the results showed that vitamin C inhibited 90% of the 64 different *H. pylori* strains examined. The results from the in vivo experiment showed a significant decrease of *H. pylori* in animals treated with vitamin C[21].

3. Discussion

3.1. *Helicobacter pylori*, salt and gastric cancer

There are several issues to be raised in regards to the GC risk factors *H. pylori* and salt. *H. pylori* infection has only recently been established as a GC risk factor. This means that only recent studies have taken *H. pylori* into account when making their research design. This is a possible confounding factor. The different types of GC do not have the same epidemiologic, clinic and molecular setting and very few studies have taken these into account when looking at nutrition and GC relation [2]. This makes the reliability of these reports questionable until further research has been done.

Salt intake varies greatly from one culture to another and in fact, salt intake may be inversely related to the use of refrigeration among people and thus socioeconomic status (which is related to GC risk) [1,3]. As mentioned earlier, since the refrigerator was introduced, GC incidence is decreasing by every decade [10]. Furthermore, researchers have encountered problems in determining salt intake since so many sources of food contain salt (salty, salted and salt-preserved foods) [1]. Some studies only takes the salt added in cooking and table salt into account though the biggest source of salt comes from salt added to processed food [1]. These studies may underestimate the intake of salt in the first place which leads to an unfair picture of the development of GC.

One study reported that gastric tissue damage occurred within a minute after administration of hypertonic NaCl solution and the number of cells in the cell cycle S-phase increased over the following 24 h [8]. However, this damage would be restored after 1 or 2 days leading to the conclusion that continuous high salt intake could be a major contributor to gastric carcinogenesis [8]. Few studies have mentioned this.
Nitrate that converts into nitrite and by nitrosation reaction to N-nitroso compounds have been shown as carcinogens in animal experiments [6]. There are few studies on dietary nitrosamines and GC. Levels of nitrosamines in food have decreased due to the addition of vitamin-C and E to cured meats which block the nitrosation reaction. However, salt have been shown to increase the levels of these carcinogenic compounds [1,4,6]. The authors suggest further research in this area to evaluate to what extent this affects GC development. Even though there is a histological difference in proximal and distal GC. Very few studies have stratified the results on the basis of GC type. Since the two types have different risk factors it seems reasonable to evaluate the two different types separately. This could have contributed to the misinterpretation of results that was not significant or even showed a decreased risk of GC with high salt intake.

Many studies investigate the relationship between salt intake and GC development in high risk areas to receive a greater sample size of GC incidence. However, this also pose a problem since there are several areas in the world where salt intake is high and GC incidence is low [for example USA and some countries in Europe]. This fact needs further research to determine why these geographical differences are seen. Host genetic factors have been proposed as a possible cause of the differences seen [5,7].

The research confirms that GC development increase with the use of salt, salty and salt preserved foods. There is also a possibility that the secondary effects of salt such as formation of N-nitrosocompounds contribute to development of GC. To limit salt intake and increase intake of fresh fruit is thus a natural step towards GC prevention.

3.2. The role of vitamin C

Vitamin C plays an important role in the human physiology and also seems to have several protective functions in terms of gastric cancer. There seems to be an association between vitamin C levels and H.pylori. In the first prospective study mentioned the results showed that the significant association between H.pylori and GC was only seen among subjects with low plasma vitamin C. In the hospital-based case-control study the association between H.pylori and GC was only seen among the subjects with low plasma vitamin C at baseline. Similar results were found in the follow-up made by Wei-cheng. The results showed that smoking and H.pylori infection are contributing factors for GC and that vitamin C has a protective effect against GC. These results indicate that the levels of vitamin C is either compromised due to the infection or has a protective effect. The results from the two nested case-control studies mentioned above also supports this theory. In this study the results also showed that the inverse association between vitamin C and GC was stronger among high consumers of red and processed meat. It is possible that the mode of action of vitamin C is through inhibiting formation of N-nitroso compounds in the gastric environment. In the hospital-based case-control study there was no difference in the presence of H.pylori between subjects in the low vitamin C intake group and the high vitamin C intake group, but in the latter group there was no significant association with GC. This indicates that vitamin C intake doesn’t effect the growth or presence of H.pylori but rather the carcinogenic effect of the bacteria. The experimental studies on the other hand all suggest that vitamin C has a direct effect on both H.pylori and GC cells.
Considering study design, they are all marred with shortcomings. In the study on Finnish male smokers by Nouraie M et al., it’s necessary to note that the subjects were all male. Furthermore, the vitamin C intake was positively associated with fruit intake and one may conclude that the separate effect of vitamin C really hasn’t been identified. Since fruits contain a lot of different compounds that can have an effect, it’s difficult to draw conclusions about the effect of vitamin C based on fruit intake. There can be internal differences concerning antioxidant concentrations depending on the season and the soil in which the fruits grow. The preparing and cooking of fruits weren’t considered either. Additionally, the use of supplements wasn’t reported.

The other prospective studies taken into account in this article measured serum or plasma vitamin C or concentration. The nested case-control in the EPIC cohort examined the effect of both dietary and serum vitamin C. Considering the collection of dietary vitamin C data this study is marred with the same flaws as the other prospective studies. The authors in this study states that dietary intakes exceeding 1000 mg/day and are associated with complete plasma vitamin C saturation and plasma vitamin C levels begin to plateau at levels above 80 μmol/l. In neither of the three prospective studies examined did the plasma vitamin C exceed this concentration, which would approve the extrapolation from plasma vitamin C to dietary vitamin C. Despite this relationship, the results from the nested case-control in the EPIC cohort did not show protective effect from dietary vitamin C. Still, this is a considerably important study as it is a vast study with a prospective approach. It’s essential to remember that behaviour change dramatically post diagnosis, including dietary behaviour. Furthermore, the populations consists of 10 European countries and includes both men and female. The case-control study is marred with several flaws due to it’s retrospective approach. Moreover, the food frequency questionnaire only included 84 items.

The inverse association between GC and either dietary vitamin C intake or plasma vitamin C seems stronger in subjects who expose themselves to reactive oxygen species such as smoking and high consumption of red meat. These results indicate that the role of vitamin C as an antioxidant and a scavenger of reactive oxygen species seems importans in terms of protective effect against gastric cancer.
References:


