Chemopreventive Approach of Garlic to the Gastric Carcinoma

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Abstract

Cancer becomes a big challenge for the current research. After leading a routine life, people are suffering from this disease. It may be due to the formation of lots of carcinogen in the environment of our regular life. Chemotherapy, radiotherapy, surgery and chemotherapeutic agents are the only ways to get relief. This disease turns people into poorer by health as well as wealth, especially in countries like India, Bangladesh, Nepal, Srilanka and many more. Some types of cancer even become curable with the help of these tools. But amongst all, gastriccancer is one which accounts high mortality rate worldwide as there are no such medications or treatments are available. This may be due to the late diagnosis of disease and the physiological position. Studies show that the effect of Helicobacter pylori is most common etiology of this disease particularly. Recently researchers focus on the anticancer action of investigational compound rather than chemopreventive activity. Very few are focusing on the preventive functions of the compounds, which can curtail the suffering of this fatal disease, by involving those compounds in our daily life. Garlic (Allium sativum) is belongs to Allium family, is one of the natural compound which shows chemopreventive activity on Helicobacter pylori induced gastric carcinoma on prolong use. On regular use of garlic or other vegetables under Allium family in-vitro demonstrates a great effect on cancer prevention activity due to the presence on thiosulfinate group. On successful continuation of this study (in vivo) can be a great gift to the civilization.

Keywords: *Gastric carcinoma, chemopreventive, Helicobacter pylori, garlic, mortality*

INTRODUCTION

Carcinoma is one of the leading causes of premature mortality worldwide [1]. Though the rapid advancement in carcinoma diagnosis and treatment modalities in past decade, have shown drastic improvement in overall survival rate of cancer patients; the clinical outcome still an unsatisfactory results due to high recurrence rate. To ensure the therapeutic success in carcinoma, a novel and efficacious treatment strategy should be developed to overcome an unmet need, which can target at various critical stages of tumour progression and also several types of carcinoma.

Among the most fatal carcinoma, gastric carcinoma is one of the leading causes of gastrointestinal cancer in the world and is the

second leading cause of cancer death worldwide [2]. The recent advancements in the molecular understanding of gastric cancer ultimately broaden up the way to discover the targeted therapies in clinical development by the researchers for this malignancy. The HER2 and COX2 over expressions along with Survivion expression are some of the findings of the recent science works which indicate their involvements in gastric carcinoma. The human epidermal growth factor receptor (HER) classified into the four receptors corresponding to a similar family, are epidermal growth factor receptor (EGFR/HER1), HER2, HER3, and HER4. These all receptors are belonging to trans-membrane glycoproteins group, but having unknown ligand and non functioning kinase characters are demonstrated by HER2 and HER3 respectively [3]. It is observed that HER2 is over expressed specially in breast cancer and gastric carcinoma also report the same in later on. In both the cases, the cell membrane is experienced with growth and transformation due to the amplification of HER2 gene followed by raised expression [4]. The Cyclooxygenase(COX) enzyme, promoter of the conversion of arachidonate to prostaglandin H2 (PGH2), is consist of at least two isozymes, i.e., constitutive COX-1 and mitogen-inducible COX-2 [5, 6]. The over expressed level of COX2 found in gastric as well as in colon cancerous cells/tissues in comparison to surrounding non-cancerous cells [7, 8]. Not only that, several cell lines derived from human gastrointestinal adenocarcinomas were also observed the presence of COX2 [9]. Survivin is a protein of IAP^4 (inhibitor of apotosis protein 4)family which is unique by its structure, localized to components of the mitotic apparatus and found during cell cycle especially in mitosis phase [10]. It has demonstrated its potentiality mainly in the inhibition of apoptosis and control of cell division [11, 12]. Survivin is found at a very low level in most human cancers or sometime in undetectable manner, correlated with reduced apoptotic index, poor prognosis, and increased risk of recurrence [13–17]. Survivin expression witnessed by gastric cancers, correlated with poor survival of patients [18–20], as well as, up-regulation of survivin is also observed in treated gastric cancer cell lines with cytotoxic drugs, indicating the chemoresistance power in gastric cancer [21]. Besides the other molecular concept, there is strong evidence that gastric adenocarcinomas are largely due to infection by the bacterium *Helicobacter pylori* which leads from gastric mucosal damage and atrophic gastritis to ultimately carcinoma [22]. It is estimated that in developing countries the risk of gastric cancer remains high with *Helicobacter pylori* carriage which is frequently ineffective by standard antibiotic regimens [23].

Though the prognosis of gastric cancer has improved year by year due to great advances in diagnostic and surgical techniques, it still remains a major cause of death throughout the world. Wanebo et al. [24], showed that the survival rate of patients with gastric carcinoma of about 5-year was only 14% mainly in the United States. Though the common treatment of patients suffering with early-stage cancer gastric cancer is only surgical resection, the 5-year survival rate is very low. This treatment includes many single agents as well as combinations to prolong survival without compromising the quality of life. Though platinum compounds, taxanes and antracyclines are used as active drugs, still uncertainty remains regarding the choice of regimen for chemotherapy as there is no internationally accepted standard of care [25].

The treatment of gastric cancer creates a great impact on the mind and lifestyle of the patient and patient's family due to the great suffering and maintenance of huge cost respectively. Due to this reason, the researchers are more focused to discover exact aetiology of gastric cancer along with the standard targeted treatment. But some of the scientists are also focusing to discover some chemo-preventive approach

against this disease which can reduce the occurrence of gastric cancer in people followed by low mortality rate and less suffering. In this context, the name of the garlic is come in front due to its preventive activity against many diseases on long term use.

MOLECULAR ASPECTS OF GASTRIC CANCER:

The molecular concepts like HER2, COX2, Survivion expressions of gastric cancer are grabbing the eyes of researchers regarding to find out the exact mechanisms.

HER2 Expression:

HER2, a biological prognostic factor along with other factors like E-cadherin, EGFR, and changes in expression of several factors including thymidilate synthase, beta-catenin, mucin antigen, p53, etc are representing a vital step to gastric adenocarcinoma by deriving from the genetic process [26]. Trastuzumab in HER2-positive tumors demonstrated the potentiality of HER2, a prognostic factor, can also be predictive response of therapy due to the molecular target in nature [26]. In 1986, the overloaded expression of HER2 protein particularly in gastric cancer was first described by immunohistochemistry (IHC) [27]. Nowadays, monoclonal antibody (HercepTest) and/or gene amplification by fluorescence in situ hybridization (FISH), are used to determine the same [28]. As per report of Gravalos et al., in a series of 166 biopsy or surgical specimens of gastric cancer patients, 13% of positive HER2 expression (IHC = 2+/FISH+ or IHC = 3+) were found along with the fact of variation of positive HER2 expression by the histology (intestinal type 16%, diffuse type 7%, unknown 14%; P = 0.276) and the primary tumor localization [25% gastroesophageal junction (GEJ) versus (vs) 9.5% gastric; P = 0.01] [29]. Lordick et al., demonstrated the significant differences of HER2 positivity by histological sub-type (intestinal 34%, diffuse 6%, mixed 20%) and as per the site of the tumor along with the fact of overexpressed HER2 more in GEJ tumour than the gastric cancer, in their study [30]. Due to the failure of some of the initial studies to find the role of HER2 as a prognostic factor in gastric cancer the issue become controversial [31, 32]. The location of HER2 gene is adjacent to the topoisomerase IIa genes which are related to the oncogene v-erb B of the avian erythroblastosis virus. High level amplification of the gene HER2 is leading to protein overexpression in the cellular membrane as it acts an oncogene in several carcinomas indicated by recent studies [33]. HER2 overexpression and/or amplification have also been observed in colon [34], bladder [35], ovarian [36], endometrial [37], lung [38], uterine cervix [39], head and neck [40], esophageal [41], and breast cancer [42] along with gastric carcinomas.

COX2 Expression:

Cyclooxygenase (COX) induces conversion of arachidonic acid to prostaglandin G2/H2 which classified mainly into two isozymes COX-1 and COX-2 [5, 6], in which COX-2 mRNA found significantly higher levels in human gastric carcinoma tissue [7]. COX-2 is also overexpressed in neoplastic tissues of colon cancers in comparison to normal tissues [8].Studies also indicated the presence of COX-2 in different cell lines of human gastrointestinal adenocarcinomas [9]. The in vitro studies on rat intestinal cells indicate that overexpressed COX-2 cause hindrance of programmed cell death [43], but the function of COX-2 regarding the cancer cells growth has not been fully established. The result of a prospective mortality study which indicates the reduced risk of fatal colon cancer on regular use of aspirin [44], raises the issue of involvement of COX2 in cancer though the exact mechanism of the inhibition whether is due to prostaglandin synthesis was unclear.

Sawaoka et al. worked on the effects of NS-398 and indomethacin regarding the growth of gastric cancer in xenografts transplanted into

athymic mice to clarify the role of COX-2 in the growth of neoplastic tissue [45]. The sulfonamide derivative NS-398 specifically inhibits COX-2 (IC50 of ~30 nM) without affecting COX-1 activity. Indomethacin has inhibitory effect on both COX-1 (IC50 = 100 nM) and COX-2 (IC50 = 900 nM) [46].An overexpressed human COX-2 expression along with COX-1 i.e. MKN45 cell-line of adenocarcinoma of the stomach [9, 47], was used by Sawaoka et al. to investigate the effects on cell replication, necrosis, and apoptosis in gastric cancer additionally [45]. The study concluded that COX2 plays an important role in the development of gastric adenocarcinoma by demonstrating the suppressed growth of tumor volume, cell replication and induced apoptosis on the human gastric cancer xenografts by COX2 inhibitors [45].

Survivin Expression:

As earlier discussed, Survivin, a member of IAP^4 protein, is showing it's potentiality in the apoptosis and cell division [11,12], of Survivin also causes the dysregulation of mitotic spindle checkpoint along with defects in microtubule assembly and function due to the antisense targeting nature which ultimately leads to cell death named as mitotic catastrophe [48–51]. Mitotic catastrophe results from aberrant mitosis, is characterized by significant increase in the percentage of abnormal nuclei, abnormally large sized nuclei, supernumerary centrosomes and failure of cytokinesis [52]. Due to these unique features, survivin becomes a promising target for cancer therapy. The studies on survivin are performed by using the transient expression method to find out the function and *in vivo* mechanism of targeting in apoptosis and cell division which is not fully understood [53,54]. *In vitro* and *in vivo* up-regulation of survivin has been demonstrated in angiogenically stimulated endothelium due to vascular endothelial growth factor and basic fibroblast growth factor whereas in quiescent endothelial cells undetectable [55- 57]. Angiogenic agents induce survivin expression because tumor angiogenesis depends on endothelial viability. In gastric cancer, expressed survivin in studies establishes correlation with poor survival of patients in 35–82.6% of cases [18–20], on the other hand up-regulated survivin in cytotoxic drugs treated gastric cancer cell lines indicates the chemoresistance character of survivin [21]. Shui et al., by using a DN mutant and by replacing the cysteine residue with alanine (Cys84Ala) at amino acid 84 i.e. the stable cell lines expressing Sur-AS cDNA or DN Sur-Mut (Cys84Ala) which can binds to the mitotic apparatus and displaces wild type survivin from polymerized microtubules [55], investigated to see the effect of constitutive suppression of survivin particularly in gastric cancer along with the effect of targeting survivin in gastric cancer treatment [58]. The positive result of this study, like induced apoptosis and *in vitro* and *in vivo* mitotic catastrophe in gastric cancer epithelial cells along with inhibition of tumor formation and angiogenesis in gastric cancer xenograft model *in vivo* by antisense (Cys84Ala)-mediated suppressed survivin also suggested that the usefulness of targeting the survivin pathway alone or with cytotoxic drugs in the treatment of gastric cancer [58].

BACTERIA INDUCED GASTRIC CANCER:

Besides the molecular concept of the gastric cancer, there is strong evidence about the bacterial involvement in gastric cancer. All the research works till date have demonstrated that the *Helicobacter pylori* are one of the aetiology of the gastric adenocarcinoma.

***Helicobacter pylori*:**

Highly genetically diverse *Helicobacter pylori* strains are found to be as freely recombinogenic populations within human hosts [59]. By using, multilocus sequence typing the genetic composition of *Helicobacter pylori* strains is generally assessed and also

compared. Studies also used this technique to find segregated strains of *Helicobacter pylori* of their corresponding human hosts [60]. Association between *Helicobacter pylori* and human beings over a period of more than 100,000-year established by these findings along with previous data, which ultimately cause less virulence of *Helicobacter pylori* over time [61-63]. For the first time, flagellated bacteria were isolated in 1982 from endoscopic biopsy specimens of patients with gastritis and peptic ulceration [64]. Since then, as an active agent of chronic gastritis, *Helicobacter pylori* are recognized. Not only that, the activities of this global micro-organism *Helicobacter pylori* is shown through the peptic ulcer disease and gastric malignancy targeting the large population which includes lower socioeconomic groups, many ethnic groups, younger ages, and certain geographical populations. This population is showing the highest risk of infection. Generally due to an unclean water sources, the infection is transmitted from person-to-person.

However, this organism remains the strongest known risk factor for gastric cancer, raising the possibility that disrupted co-evolution between *Helicobacter pylori* and human beings may affect pathogenesis. To determine the effects of co-evolutionary relationships (genetic variations) between *Helicobacter pylori* and human beings on the development of intestinal-type gastric cancer, Kodaman et al used multilocus sequence type and single nucleotide polymorphism analyses to investigate [66]. The predicted risk for intestinal-type gastric cancer is mainly a specific interaction between microbial and human genetic ancestries. Depending on the interactions between host (human) and pathogen (*Helicobacter pylori*) ancestries along with the genetic mismatch, the severity of gastric injury is dependent whether it will cause gastritis or cause cancer [65, 66]. The evidence of involvement of more granular interactions between host and pathogen genotypes to alter the risk of gastric cancer is also there. To investigate the virulence factor of *Helicobacter pylori*, the intensively studied and well-characterized factor is the cag pathogenicity island (PAI). The presence of the cag PAI in strains increases the risk for distal gastric cancer in comparison to strains that are lacking this locus [67]. In case of human genetics, the risk of gastric cancer among *Helicobacter pylori*–infected persons is determined by the specific polymorphisms in genes that encoding inflammatory cytokines [68].Though the persons infected with cag^+ strains were reported more polymorphisms in IL1, IL10, or TNF, in comparison to H pylori–infected population in case of distal gastric cancer [69], but the H pylori infected persons possess the presence of type s1/m1 vacA alleles, another strain-specific genetic locus, which are likely to trigger gastric cancer along with hypochlorhydria. Not only that, the chances of gastric cancer can increase up to 87-fold over baseline when the vacA alleles or cag genotype combined with high-risk host genotypes of *Helicobacter pylori*.[70].

Reason for High Mortality Rate:

Gastric cancer becomes the second leading cause of cancer as per mortality rate [71]. The high mortality rate is due to many reasons which include the physiological position of stomach and the sign and symptoms of the disease. In generally, the stomach lies between the oesophagus and the duodenum, whereas the top of the stomach lies against the diaphragm. The exact location of the stomach is the left upper part of the abdominal cavity. Pancreas is just situated behind. This complicated position of stomach makes physician as well as researcher confused to diagnosis and to design for targeted therapy respectively. The common sign and symptoms of the gastric cancer includes discomforts or pain in stomach area, nausea and vomiting, difficulty in swallowing, weight loss, feeling bloated after a small meal and sometimes accompanied with vomiting blood or having blood in the stool are very

similar to common GIT problem. Due to this confusion, patients having stomach cancer are leading to late diagnosis which ultimately increases the risk of mortality. Not only that, the availability of fewer treatments or medications which aren't clinically proven till date to serve at later stage is also a great reason.

Chemotherapeutic Approach/Treatment:

In case of gastric carcinoma, the availability of proper treatment is almost none. Surgical resection followed by radiation and chemotherapy are the most common therapy for the late diagnosed gastric cancer. But in most of the cases, patients are diagnosed at an unresectable stage where the systemic chemotherapy becomes the only treatment option. Still now there is no such internationally accepted standard of care is available though many single agents and combinations are actively used which doesn't improve the survival rate [29].

As discussed earlier, HER2 overexpression is one of the aetiology of gastric cancer. Gravalos et al. had shown in experimental models, suppressing power of the trastuzumab an anti-HER2 therapy in growth of human gastric cancer with HER2 overexpression in vitro and in vivo [29]. The results of this study along with others, are exploring the potential of anti-HER2 therapies in gastric cancer patients along with better knowledge of the efficacy and tolerance of trastuzumab-based therapy in HER2-positive gastric cancers.

Chemotherapeutic Versus Chemopreventive Approach:

This therapy is seems to be very painful, costly and long lasting to the patient and their family. During the journey of the different phases of this therapy patients lose their physical as well as mental strength due to the hectic and painful procedure. Not only that, the families of the patients are also losing their hope and they also defeated against the cost of therapy. To maintain such a huge cost on long term became difficult for the people belongs to poor economic country. Most importantly, after suffering and bearing the load of pain and cost, families are witnessed with the death of the patients in maximum cases.

We are very familiar to the quote "Prevention is better than cure" which suggest people to adopt some good and healthy lifestyle in our daily routine to avoid major illness. From this concept, the vaccination against some major disease has been developed. This preventive approach is well recognized and accepted in all over world due to the easy affordability and painless technique. Similarly, in major diseases like cancer chemopreventive approaches are highly acceptable to avoid the pain of suffering from the disease and the unbearable cost. People are already owned some measures in their life which includes diet, lifestyle, exercise and environmental factors, in one word developing some healthy habits which is also easily affordable by anyone. As a result it can be great blessing for society against the curse named carcinoma.

Figure 1: A Chemical structure of Thiosulfinate B: Chemical structure of Allicin

GARLIC: AN ALLIUM GROUP VEGETABLE:

The antibacterial/antibiotic activity *in vitro* of raw juice of garlic *(Allium sativum* L.), a member of *Allium* group and its preparations reviewed by Reuter and co-workers [72], have showed activity against both the Gram-negative and Gram-positive bacteria which includes *Escherichia coli, Pseudomonas, Salmonella, Candida, Klebsiella, Bacillus subtilis, Staphylococcus aureus* and also prevent toxin production by microorganisms. The mechanisms like modulation of SH enzymes, inhibition of RNA synthesis, and partial inhibition of DNA and protein synthesis can be the reasons behind the antibiotic activity of garlic [72]. The thiosulfinate group is the core constituent of *Allium* group. Incorporation of selective removal of the thiosulfinates (Fig:1) either by solvent extraction process or by reaction with cysteine, along with prevention of their formation by inhibition of alliinase, Reuter et. al. demonstrated the antibiotic activity of garlic due to allicin (Fig: 2) and the other thiosulfinates [72]. Here the mechanism behind is oxidization of SH groups of bacterial enzymes which is followed by the retardation of bacterial growth [72].

Not only the antibiotic property, garlic can easily inhibit the diarrhoea causing enterotoxic *E. coli* strains and other pathogenic intestinal bacteria more than those that constitute the normal intestinal flora. Garlic also showed partial or total synergistic activity mainly against aerobic bacteria when used in combination with antibiotics. In terms of resistance power, lack of resistance has been observed repeatedly to garlic along with activity against those strains which are already become resistant to antibiotics.

Apart from the antibacterial activity, garlic has showed its effect on major organs like kidney leading to UTI, lungs followed by chronic bronchitis and also improve the immunization power and insulin production in the individuals. Reduction of blood sugar, blood pressure and cholesterol is also reported as the valuable physiological role of garlic along with its antioxidant property.

Garlic in Gastric Cancer:

As earlier discussed, *Helicobacter pylori* is a one of the common cause of having stomach cancer and all antibacterial agents were failed against it. According to studies, the incidence of stomach cancer is inversely proportional with a high intake of *Allium* (Garlic*)* vegetables [73, 74]. The work of Shivam et al. was focused preliminary on finding of the preventive activity of garlic extract against *Helicobacter pylori* due to its strong antimicrobial property by performing MIC test at a concentration which is not inhibitory to *Staphylococcus aureus* [75]. *Garlic* shows strong activity against *Helicobacter pylori* in lower concentration due to the presence of allicin, an active thiosulfinate group. Amongst the *Allium* group, Garlic was chosen by the team to see the susceptibility,, due to its easy availability, nontoxic nature, difficulty to develop resistance. Not only that, Garlic gets recognition as standard food item which can be consumed on daily basis over long periods of time easily [73,74]. Shivam and team demonstrated the selective potency of garlic extract against *Helicobacter pylori* compared with *Staphylococcus aureus,* by keeping the lower minimum inhibitory concentration (MIC) for *Helicobacter pylori* than for *Staphylococcus aureus* [75]. Generally, the inhibitory concentration of the garlic extract >1 mg/ml was used by the most of the researcher [72] though the Shivam et al. used different concentration ranging from 40µg/ml to 160µg/ml against *Helicobacter pylori* where

the lower concentration showed the effect. The thiosulfinate group shows its antibacterial activity by oxidizing the SH groups of antimicrobial enzymes followed by retardation of growth [72].Differences in cell membrane composition in several species of bacteria like 20% lipid in *Escheria coli* and 2% in *Staphylococcus aureus* cause susceptibility to thiosulfinates [75]. Though the lipid contain of *Helicobacter pylori* is unknown, the characterization of lipid composition has been done [76]. These differences ultimately results variation of thiosulfinates membrane permeability followed by the susceptibility to these organosulfur compounds.Due to highly effectivity against *Helicobacter pylori* along with other microorganisms, of thiosulfinates researchers believed the relation between the lower risk of stomach cancer in individuals with a high Allium vegetable intake.

Not only *Helicobacter pylori* induced gastric cancer, garlic showed its activity in molecular level too.The generation of reactive oxygen species (ROS) is an importan biochemical processes, but in little amount as it is not harmful. The low level ROS is required in processes like immunity, intracellular messaging and defence against microorganisms. Simultaneously, it can cause induction of apoptosis, cell cycle arrest along with pathogenesis of gastric malignancies at higher concentration [77]. Wang et al. showed that antioxidative and antiproliferative activities of garlic by increasing the activity of serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in tumor-bearing animals whereas the relation with ROS is inverse in case of induction of apoptosis. Components like S-allyl cysteine (SAC) and S-allylmercapto-L-cysteine (SAMC) in fresh garlic are converted into stable and water-soluble Organosulfur compounds (OSCs) on aging; possess high radical scavenging activity, which directly or indirectly remove ROS [77].

CONCLUSION:

It can be concluded that, garlic and Allium vegetables can be intervened as a potent chemopreventive agent against Gastric carcinoma among the populations at high risk particularly where antibiotic resistance and the risk of reinfection are high. Due to its easy availability and recognition as standard food items which finally lead to its consumption on regular and long term basis, nontoxicity and low cost; which will curtail the pain suffering and costing of the patient, disease and treatment respectively the garlic can be used. But the long path to go as all the respective data are only limited to the in-vitro studies. It can be happen if only more in-vivo studies followed by clinical trial can be performed & the results of those will prove the same.

But one more conclusion also can be drawn, that if researchers are focusing on chemopreventive activity of different compound instead of chemotherapeutic activity, society will be benefitted as it will help to live a healthy and balanced life.

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