Cancer is currently the second leading cause of death in the world behind cardiovascular diseases. It is estimated that more than 1.6 million new cases of cancer are diagnosed in every year (American Cancer Society, 2012). Cancer is characterized by the proliferation of abnormal cells that fail to respond correctly to normal regulatory mechanisms. Carcinogenesis, a term used to describe cancer development, is a multiple-step process consisting of initiation, promotion, and progression of uncontrolled cells. At the initiation step, damage to DNA occurs. Finally, cells begin to proliferate and expand into abnormal cells during the promotion step and during the progression step, further changes occur to these abnormal cells leading to formation of malignant cells (Klaunig J, et al., 2004).

The incidence of gastric cancer is different throughout the world and 60% of deaths from gastric cancer occur in developing countries (Forman D, et al., 2006; Liu T S, et al., 2008). Cancers of the gastrointestinal tract, including esophageal, stomach, liver, colon, and pancreas are responsible for approximately 3 million new cases and over 2 million deaths each year (Hamilton S R, et al., 2000). Malignancies of the G.I. tract are relatively resistant to radiation therapy while chemotherapy has modest benefit so an effective therapy still remains elusive in the treatment of gastroduodenal ulceration (Luck G D, 1998). Early diagnosis of human gastric cancer or tumor recurrence is primarily based on endoscopy, biopsy and pathological examination. Endoscopy is a widely used method for detecting early stages of gastric cancer (Tashiro A, et al., 2006; Lu X et al., 2008).
Pathology

The primary epithelial tumor of the stomach is the adenocarcinomas develop from the stomach mucosa, usually maintaining glandular differentiation. Most common tumors of the stomach are the squamous cell carcinomas, and the adenosquamous carcinomas, combining characteristics of both the adenocarcinoma and the squamous cell carcinoma to approximately equal extent. Undifferentiated carcinoma lacks any differentiated features and does not fit into any of the above categories. Gastric carcinomas can be classified according to their localization in the stomach. The antral-pyloric region of the stomach is the most common site of stomach cancer, and carcinomas of the body or corpus are located along the greater or lesser curvature. Cancers of the cardia are often unable to be distinguished from cancers of the gastroesophageal junction, and are believed to be a separate entity, probably originating from the distal oesophagus.

The diagnosis of gastric cancer is often delayed by the lack of early symptoms, with early gastric cancer causing non-specific gastrointestinal complaints, such as dyspepsia, in only 50% of patients. Up to 90% of Western gastric cancer patients first present with advanced carcinomas, which have more serious symptoms such as abdominal pain, bleeding, vomiting, or severe weight loss. Endoscopic screening is considered to be the most sensitive and specific diagnostic test for gastric cancer. Dysplasia may present as a flat lesion or exhibit polypoid growth, with depressed, reddish or
discoloured mucosa. Endoscopic detection of changes in colour, relief, and architecture of the mucosal surface enables the classification of gastric cancers according to their macroscopic growth pattern. Early gastric cancers may feature protruded (Type I), elevated (Type IIa), flat (Type IIb), depressed (Type IIc) or excavated (Type III) growth (Hamilton S R, et al., 2000), whereas advanced gastric carcinomas are classified into polypoid (Type I), fungating (Type II), ulcerated (Type III) or infiltrative (Type IV) growth patterns (Borrmann R, 1926; Hamilton S R, et al., 2000). Type II or III advanced gastric cancers are commonly ulcerating, and the risk of penetration of the submucosa is highest in early gastric cancers with a depressed growth pattern (Type IIc), and in infiltrative advanced gastric carcinomas (Type IV). The superficial spread of Type IV infiltrative (diffuse) tumours through the mucosa and submucosa result in flat, plaque-like lesions, which may exhibit shallow ulcerations. Serosal, lymphatic, and vascular invasion and lymph node metastases are most frequent in the diffusely growing.

**Epidemiology**

Gastric cancer is one of the most common cancers worldwide, ranking fourth in overall frequency, and accounting for over 870,000 new cases and over 650,000 deaths annually in world. (Stewart B W, et al., 2003). Mortality from gastric cancer is second only to lung cancer. Gastric cancer occurs more frequently in men than in women, with the estimated number of new cases worldwide being 558,000 for males and 317,000 for females, respectively.
accounting for 5.5% and 3.1% of all malignancies, excluding skin cancer (Hamilton S R, et al., 2000). The geographic distribution of gastric cancer varies from an annual incidence of more than 300,000 new cases in the more developed regions, to nearly 550,000 new cases per year in the developing or less developed regions of Africa, Latin America and the Caribbean, Asia (excluding Japan), Micronesia, Polynesia and Melanesia. In high risk areas, the intestinal type adenocarcinoma is more frequent, whereas the poorly differentiated diffuse type carcinoma predominates in low risk areas.

The incidence and mortality rates of gastric carcinoma are steadily declining. However, due to the aging population, the absolute number of new cases per year is increasing (Munoz N, et al., 1971; Hamilton S R, et al., 2000). Below the age of 30, the incidence of gastric carcinoma is extremely rare, but thereafter rises quickly and continuously, with the oldest age groups having the highest rates. In males, the intestinal type is more common than the diffuse type and the incidence rises faster with age, whereas the diffuse-type mainly impacts younger individuals, frequently females. A decline in incidence of the intestinal-type carcinomas is largely responsible for the decline in overall incidence rates (Kaneko s, et al., 2001; Henson D E, et al., 2004), and has been correlated with the corresponding decrease in prevalence of H. pylori infection (The Eurogast Study Group, 1993; Konturek P C, et al., 2003).

Both gastric cancer and H. pylori infection affect patients from low socioeconomic backgrounds, associated with low social class, poor education,
low hygiene standards, a diet lacking fresh fruit and vegetables, but rich in starch and preserved meats, and atrophic gastritis. Indeed, the distinctive epidemiological characteristics of gastric cancer, in particular, the regional differences and chronological changes in incidence may be, in part, related to H. pylori infection (Nagel G, et al., 2007). However, the incidence of the diffuse-type carcinoma may be increasing (Craanen M E, et al., 1992; Henson D E, et al., 2004), which is worrying given that these types of tumors have a worse prognosis (Blok P, et al., 1997). An increase has also been observed for cancers localized to the gastro-oesophageal junction, some probably originating from the distal esophagus caused by gastro-oesophageal reflux (Yamada Y, et al., 1989). Cancers of the cardia and gastroesophageal junction are conspicuously increasing in incidence and frequently exhibit a different pathogenesis to non-cardia carcinomas.

Pathogenesis

The pathogenesis of gastric cancer involves multiple risk factors including dietary, infectious, occupational, genetic and preneoplastic risk factors, most of which act on the gastric mucosal microenvironment over a prolonged time period. The resultant sequential changes in the gastric mucosa that precede the development of invasive cancer are known as the “precancerous cascade”, first described in 1975 (Correa P, et al., 1992), where normal gastric mucosa is transformed by chronic atrophic gastritis and develops multifocal atrophy and intestinal metaplasia, followed by the appearance of dysplasia and finally invasive carcinoma.
Past research has concentrated on the identification of the complex etiology of environmental and genetic risk factors, which may influence the initiation, promotion, and progression of gastric cancer (Stadtländer C T, et al., 1999; Chan A O, et al., 2001; Correa P, 2002; Kelley J R, et al., 2003).

**Histological Classification**

Various systems have been applied to the classification of gastric carcinomas, including the WHO (Hamilton S R, et al., 2000), Ming (Ming S C, 1977), Lauren (Lauren P, 1965), and Goseki (Goseki N, et al., 1992) classifications. The clinical significance of these classifications is limited, with only the Lauren and perhaps the Goseki classifications providing prognostic assessments (Alekseenko et al, 2004). The TNM staging of the gastric carcinoma, according to the guidelines set out by the International Union Against Cancer (Wittekind C et al., 2002), is the most important prognostic factor in clinical practice (Alekseenko S A, et al., 2004). However, the Lauren classification has been the most successful system, as it defines two distinct histological entities, which clearly exhibit different clinical and epidemiological characteristics, even in advanced gastric cancers (Satoh et al, 2007).

In the Lauren classification (Lauren P, 1965), intestinal-type carcinomas maintain the glandular phenotype, with well- to moderately-differentiated tumours forming identifiable glands, often with poorly differentiated tumour cells at the invasive front. Typically arising on a background of intestinal metaplasia, these tumours exhibit an intestinal,
gastric and gastrointestinal mucinous phenotype. Diffuse-type carcinomas form no or very few glandular structures, instead usually infiltrating the gastric wall, appearing diffusely distributed as small, round single cells or poorly cohesive cell clusters. They may resemble signet-ring cells, and may contain small amounts of intestinal mucin. Additionally, mixed tumours exhibit both intestinal and diffuse characteristics, and undifferentiated tumors are classified as indeterminate. The natural history of gastric carcinoma, in particular the association with environmental factors, incidence trends, and precursor lesions, is often evaluated with respect to the Lauren classification.

**World Health Organization (WHO) Classification of Gastric Cancer**

The World Health Organization (WHO) classification issued in 2010 appears to be the most detailed among all pathological classification systems. It is remarkable that the WHO classification includes not only adenocarcinoma of the stomach but also all other types of gastric tumors of lower frequency (Flejou JF, et al., 2011). The gastric adenocarcinoma type is divided into several subgroups including papillary, tubular, mucinous and mixed carcinoma, which can be compared to the indeterminate type in the Lauren classification. The poorly cohesive carcinoma type includes the signet ring cell carcinoma. All other classified gastric adenocarcinomas can be designated as uncommon because of their minor clinical relevance. In the WHO classification, the most common type of gastric cancer is the tubular adenocarcinoma, followed by the papillary and mucinous types. The signet
ring cell carcinoma accounts for approximately 10% of gastric cancers and is defined by the presence of signet ring cells in over 50% of the tumor (Flejou J F, et al., 2011; Werner M, et al., 2001). The prognosis of the signet ring cell carcinoma is controversial. Most authors have described a worse prognosis for the signet ring cell carcinoma compared to other subtypes of gastric cancer (Ribeiro M M, et al., 1981, Hass H G, et al. 2011). Recent studies indicate that, on the contrary, signet ring cell carcinoma of the stomach does not differ in prognosis from the other types of gastric cancer (Hass H G, et al., 2012). Furthermore, signet ring cell carcinoma was shown to have an irregular uptake of \( {\text{18F}} \)-fluorodeoxyglucose during positron emission tomography radionuclide imaging; consequently, this tumor as well as any metastases cannot be detected reliably (Alakus H, et al., 2010). Patients with a papillary adenocarcinoma experience a poor prognosis, a tendency for metastatic disease, a higher age at diagnosis and location in the upper third of the stomach (Yasuda K, et al., 2000). Another study that employed the previous WHO classification found that poorly differentiated and mucinous adenocarcinomas have a worse prognosis than the papillary and tubular subtypes. In the same study, the WHO classification appeared to be an independent prognostic factor (Zheng H C, et al. 2010). Kawamura H, et al., (2001) also found a poor prognosis associated with mucinous adenocarcinoma, which suggests a link with advanced stage and metastatic disease. However, unlike most common types of gastric malignancies, the WHO classification is more widely used for studies of infrequent types of
gastric cancer. For adenosquamous carcinomas of the stomach, a poor prognosis and a case of simultaneous gastric adenocarcinoma are described (Toyota N, et al., 1996; Faria G R, et al., 2010; Su J S, et al., 2013). Most of the infrequent types of gastric malignancies are described in case reports, so a systematic investigation of their prognoses is not readily available. As the previous WHO classification was renewed in 2010, it is expected that more gastric cancer studies that refer to the most recent WHO classification will be conducted in the near future. An indication for the significance of the WHO classification can be seen in a similar Japanese classification system. Although the Japanese classification divides the common types of gastric adenocarcinoma into additional subtypes, (e.g., tubular adenocarcinoma is divided into well differentiated and moderately differentiated adenocarcinoma), a dependence on the WHO classification system is evident (Japanese Gastric Cancer Association, 2011). This particular subdivision of tubular adenocarcinoma was based on differences in the submucosal invasion rate, lymph node metastasis and size of the lesions (Fujii T, et al., 1994).
Lauren and World Health Organization classification

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<tr>
<th>Intestinal type</th>
<th>Diffuse type</th>
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<td>• Papillary adenocarcinoma</td>
<td>• Signet-ring cell carcinoma and other poorly cohesive carcinoma</td>
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<td>• Tubular adenocarcinoma</td>
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<td>• Mucinous adenocarcinoma</td>
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<td><strong>Diffuse type</strong></td>
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<th>Intermediate type</th>
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<td>• Mixed carcinoma</td>
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<td>• Adenosquamous carcinoma</td>
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<td>• Squamous cell carcinoma</td>
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<td>• Hapaloid adenocarcinoma</td>
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<td>• Carcinoma with lymphoid stroma</td>
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<td>• Choriocarcinoma</td>
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<td>• Carcinosacoma</td>
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<td>• Parietal cell carcinoma</td>
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<td>• Malignant rhabdoid tumor</td>
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<td>• Paneth cell carcinoma</td>
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<td>• Undifferentiated carcinoma</td>
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<td>• Mixed adeno-neuroendocrine carcinoma</td>
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<td>• Endodermal sinus tumor</td>
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<td>• Embryonal carcinoma</td>
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<td>• Pure gastric yolk sac tumor</td>
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<td>• Oncocytic adenocarcinoma</td>
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**Prognosis**

The prognosis of gastric cancer depends on various pathological factors, such as the macroscopic type, the depth of invasion, cancer-stromal relationship, histological growth pattern, lymph node involvement, lymphatic invasion, vascular invasion and tumour site with the main prognostic factors being the TNM staging, along with the presence and extent of lymph node metastases (Yokota T, et al., 2004). Diagnostic improvements and advances in treatment options have improved the long-term survival of early gastric
cancer patients. Prognosis is correlated with the tumour staging, worsening as the degree of infiltration increases (Lello E, et al., 2007). The 5-year survival rates in stages Ia, Ib, II, IIIa, and IIb/IV are 91%, 64%, 27%, 18%, and 0%, respectively with the survival rate of patients with early gastric cancers invading but limited to the mucosa or submucosa being 90-100%, compared to 60-80% for tumours reaching the muscularis propria, and 41-50% for tumours limited to the subserosa or serosa (Antonioli D A, 1994; Yoshikawa K, et al., 1985; Tanaka K, et al., 2004; Lello E, et al., 2007). However, the prognosis of advanced gastric cancer remains poor, with survival rates lower than 23% (Tanaka K, et al., 2004) and rarely exceeding 15% (Stewart B W, et al., 2003). The depth of infiltration correlates with the presence of lymph node metastases, and the presence of regional lymph node metastases reduces the 5-year survival rate of early gastric cancer patients from 90% to 70% in tumours invading the submucosa (Antonioli D A, 1994; Inoue K, et al., 1991).

The lymph node status and the ratio of metastasispositive/metastasis-negative lymph nodes are the strongest markers of gastric cancer prognosis (Ichikura T, et al., 1999; Yokota T, et al., 2004), and the N-ratio (metastatic/examined lymph nodes) has been validated as an independent prognostic factor in a large multi-centre series, even where less than the recommended 15 lymph nodes have been examined (Marchet A, et al., 2007).

The 5-year survival rate for patients with metastases in 1-6 lymph nodes is 44%, and drops to 30% for 7-15 lymph node metastases, ending
with 11% for more than 15 lymph nodes metastases. The N-ratio classifications 0, 1, 2, and 3 exhibit 5-year survival rates of 83.4%, 66.3%, 46.8%, and 19.0%, respectively. Unfortunately, most patients presenting with advanced gastric cancer already have lymph node metastases.

Other prognostic factors include lymphatic and vascular invasion, both being associated with lower survival rates (Hamilton S R, et al., 2000; Yokota T, et al., 2004), and the histological classification, whereby diffuse type (Lauren classification) and mucous-rich (Goseki classification) tumours may predict a worse prognosis (Martin I G, et al., 1994; Songun I, et al., 1999; Hamilton S R, et al., 2000.)

**Causes of Gastrointestinal carcinoma**

**Helicobacter pylori**

Helicobacter pylori, a Gram-negative microaerophilic, spiral bacterium found in the gastric mucosa in patients with severe gastritis and chronic atrophic gastritis, has been recognized as an important risk factor for gastric cancer (Catalano V, et al., 2009; Houghton J, et al., 2005). The results of several meta-analyses concluded that H. pylori infection is associated with an approximately two-fold increased risk of developing gastric cancer (Eslick G D, 2006). In a prospective study involving 1526 Japanese patients who had duodenal ulcers, gastric ulcers, gastric polyps or non-ulcer dyspepsia, 2.9% of H. pylori infected patients subsequently developed gastric cancer while none of the uninfected patients developed tumors (Uemura N, et al., 2001). In 1994, the International Agency for Research on Cancer categorized H. pylori...
as a “Group 1 human carcinogen” based on a plethora of studies (Schistosomes. 1994).

Currently, approximately 50% of the world’s population is infected by H. pylori. The prevalence of H. pylori infection varies markedly in different countries in Asia with seroprevalence rates higher in developing countries than in industrialized, developed nations (Fock K M, et al., 2010) The identification of H. pylori as a risk factor for gastric carcinogenesis has stimulated extensive research on the mechanisms by which H. pylori induces carcinogenesis. A combination of a virulent organism, a permissive environment, and a genetically susceptible host is considered essential for H. pylori-induced gastric cancer. H. pylori has been suggested to trigger a cascade of events that promote the sequential progression of normal gastric epithelium through atrophic gastritis, intestinal metaplasia, and dysplasia to carcinoma (Kim S S, et al., 2011; Amieva M R, et al., 2008; Hofman P, et al., 2004) The bacterium secretes several products that cause gastric mucosal damage such as urease, protease, phospholipase, ammonia, and acetaldehyde. H. pylori disrupt gastric barrier function via urease-mediated myosin-II activation (Wroblewski L E, et al., 2009).

Generation of oxidative stress is recognized as a virulence factor in H. pylori-infected hosts. H. pylori infection induces the production of reactive oxygen and nitrogen species and suppresses the host antioxidant defense mechanisms, leading to oxidative DNA damage. However, H. pylori, which is endowed with a variety of antioxidant enzymes is spared from oxidative
stress and the damage is solely restricted to the gastric mucosa of the susceptible host (Suzuki H, et al., 2012). H. pylori although not directly mutagenic, has been suggested to favor the formation of mutagenic substances through inflammatory mediators or by impairing the mismatch repair pathway (Yao Y, et al., 2006). Kim s s, et al., (2011) demonstrated that H. pylori infection promotes gastric carcinogenesis by increasing endogenous DNA damage whilst decreasing repair activities and by inducing mutations in the mitochondrial and nuclear DNA. Aberrant DNA methylation induced by H. pylori infection has been found to be a significant risk factor for gastric cancer (Perrin D, et al., 2010). Epidemiological evidence suggests that H. pylori strains containing the cag pathogenicity island (cagPAI) are more virulent. The cagPAI is a 40-kb genome segment that encodes approximately 30 genes including the cytotoxin-associated gene A (cagA). The virulent cagA positive strains increase the risk of non-cardia gastric cancer of both intestinal and diffuse types, but not the risk of cardia cancer. The CagA protein is delivered into gastric epithelial cells where it undergoes tyrosine phosphorylation by SRC family kinases. Phosphorylated CagA specifically binds to and activates SHP2, a phosphatase that transmits positive signals for cell growth and motility. Thus H. pylori acting via cagA activates growth factor receptors, increases proliferation, inhibits apoptosis, and promotes invasion and angiogenesis (Hatakeyama M, et al., 2004).

Gene expression profiling of gastric antral mucosa samples from H. pylori infected patients by microarray analysis followed by quantitative real-
time PCR assays have revealed differential expression of 38 genes, indicating that H. pylori infection leads to evasion of host defense, enhanced inflammatory and immune responses, activation of NF-κB and Wnt/β-catenin signaling pathways, perturbation of metal ion homeostasis, and induction of carcinogenesis (Yang Z M, et al., 2012)

**Epstein-Barr virus infection**

The human herpes virus 4, or Epstein-Barr virus (EBV), is an icosahedral herpes virus containing double stranded DNA that has been connected with gastric cancer. The EBV has been classified as a Group I carcinogen by the WHO and IARC, and is ubiquitous in all human populations. EBV is the cause of Burkitt’s lymphoma, sino-nasal angiocentric T-cell lymphoma, Hodgkin’s disease and nasopharyngeal carcinoma (IARC, 1997). EBV-associated carcinomas are found in all geographic regions (Stadtländer et al., 1999), and are approximately three-fold more frequently found in Japanese than in American populations (Watanabe et al, 1997). EBV is associated with both intestinal- and diffusetype gastric cancers (Shibata D, et al., 1992), but may be more prevalent in the male than in the female (Tokunaga et al, 1993). The mechanism of EBV-mediated gastric carcinogenesis is as yet unclear. Virus replication occurs in pharynx and salivary gland epithelial cells. The subsequent infection of lymphoid B-cells is mediated by the interaction of the gp350 viral envelope glycoprotein and CD21, the C3d complement component CR2 (IARC, 1997). The viral glycoproteins gp85, gp25 and gp42 are involved in host cell binding and viral
envelope fusion, with the virus persisting in a latent state until triggering of the host cell results in shedding of infectious virus particles (IARC, 1997). Up-regulation of p53 is rarely observed in EBV-positive carcinomas, but found in over 30% of EBV-negative carcinomas (Ojima H, et al., 1997; Chang M S, et al., 2005) and p27 loss, p16 loss, cyclin D1 expression and NF-κB nuclear positivity are found more frequently in EBV-positive gastric carcinomas (Chang M S, et al, 2005). Despite the association of EBV infection with the development of gastric carcinoma, there is no correlation with bcl-2 expression and p53 accumulation (Gulley et al, 1996), leading to the conjecture that EBV induces gastric carcinomas via different mechanisms than EBV-negative carcinomas (Ojima H, et al., 1997).

**Dietary factors**

A survey of literature on the role of diet in the pathogenesis of gastric cancer using Pub Med as a search platform has revealed over 2000 epidemiological and experimental studies. Populations at high risk for stomach cancer have been shown to consume diets rich in starch and poor in protein quality, and are not inclined to eat fresh fruits and vegetables. Both high starch and low protein diet may favor acid-catalyzed nitrosation in the stomach and cause mechanical damage to the gastric mucosa (Krejs G J, et al., 2010; Berretta M, et al., 2012; Tsugane S et al., 2007). Using an ecological approach, Park B, et al., (2011) found a negative association between refrigerator use, fruit intake, and gastric cancer mortality and positive
associations between salt/ sodium intake and gastric cancer mortality and incidence in Korea.

Both epidemiological and experimental studies strongly support the role of excessive salt intake in gastric carcinogenesis. D’Elia L, et al.,(2012) reported a direct correlation between dietary salt intake and risk of gastric cancer with progressively increasing risk across consumption levels based on a meta analysis of prospective studies. Consumption of large amount of salted fish, soy sauce, pickled vegetables, cured meat and other salt-preserved foods enhances H. pylori colonization, and increases the risk of gastric cancer through direct damage to the gastric mucosa resulting in gastritis. Salt is also known to induce hypergastrinemia and endogenous mutations, promoting epithelial cell proliferation which eventually leads to parietal cell loss and gastric cancer progression (Tsugane S, et al., 2004 Wang X Q, et al., 2009). Reports from this laboratory as well as by other workers have demonstrated that saturated sodium chloride (S-NaCl) promotes the development of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced rat gastric carcinomas (Velmurugan B, et al.,2005; Kim D J, et al.,1997) Dietary nitrates are found either naturally in foods such as cabbage, cauliflower, carrot, celery, radish, beets, and spinach or added during preservation. In addition, the nitrate content of fertilizers, soil, and water also contribute to dietary nitrate. Nitrite, nitrate, and nitro satu ing agents can be synthesized endogenously by reactions mediated by bacteria and activated macrophages. Nitrosation of a number of naturally occurring guanidines and L-arginine-containing polypeptides
produces mutagenic compounds. Dietary nitrate is converted to carcinogenic N-nitroso compounds (NNC) by gastric acid thereby increasing gastric cancer risk. Small quantities of preformed NNC and nitrosamines may also be present in some foods including cured meats, dried milk, instant soups, and coffee dried on direct flame (Suzuki H, et al., 2005; Mitacek E J, et al., 2008; Liu C, et al., 2008)

In addition to specific components of the diet, certain cooking practices are also associated with increased risk of gastric cancer. These include boiling of meats, roasting, grilling, baking, and deep frying in open furnaces, sun drying, salting, curing, and pickling, all of which increase the formation of NNC. Polycyclic aromatic hydrocarbons such as benzo(a)pyrene formed in smoked food have been incriminated in many areas of the world with high stomach cancer rates (Jedrychowski W, et al., 2003; Wogan G N, et al., 2004)

**Lifestyle**

Alcohol, a gastric irritant is an important risk factor for gastric cancer. Zaridze D, et al., (2000) have reported an increased risk of stomach cancer in men and women who regularly consume strong alcoholic beverages. A direct correlation was observed between consumption of alcohol and tobacco and the risk of gastric cancer in a population-based prospective cohort study (Sjodahl K, et al., 2007). A study from this laboratory demonstrated a positive correlation between alcohol consumption and cigarette smoking with the blood lipid profile in gastric cancer patients (Manoharan S, et al., 1997). The
European Prospective Investigation into Cancer and Nutrition (EPIC) project found a significant association between the intensity and duration of cigarette smoking and gastric cancer risk (Gonzalez C A, et al., 2003). Smoking history was found to be a significant independent risk factor for death from gastric cancer in patients who had undergone curative surgical resection (Smyth E C, et al., 2012). Smoking is known to decrease prostaglandins that maintain gastric mucosal integrity (Mc Cready D R, et al., 1985). Tobacco smoke has been reported to induce the development of precursor gastric lesions such as gastritis, ulceration, and intestinal metaplasia. Smokers tend to have a higher incidence of \textit{H. pylori} infection and gastroduodenal inflammation than non-smokers (Gonzalez C A, et al., 2010).

**Family history**

Gastric cancer is a known manifestation of inherited cancer predisposition syndromes similar to hereditary nonpolyposis colon cancer and Li-Fraumeni syndrome. According to the OMIM database, 90% of gastric cancers are sporadic, whereas 10% are hereditary. The first documented report of familial predisposition to gastric cancer was described for Napoleon Bonaparte’s family with Napoleon, his father, grandfather, brother, and three sisters, all dying of stomach cancer at a relatively early age (Bevan S, et al., 1999). The Scandinavian twin study in the Swedish, Danish, and Finnish twin registries found an increased risk of stomach cancer in the twin of an affected person (Lichtenstein P, et al., 2000). Family members usually share the same environment and have similar socioeconomic status. These risk factors act
independently or in conjunction with genetic factors thereby increasing the risk of stomach cancer.

**Occupations**

A positive correlation has been recognized between increased stomach cancer risk and a number of occupations including mining, farming, refining, and fishing as well as in workers processing rubber, timber, and asbestos (Krstev S, et al., 2005; Straif K, et al., 2000). Occupational exposure to dusty and high temperature environments such as in cooks, wood processing plant operators, food and related products machine operators was associated with a significant increased risk of gastric cancer of the diffuse subtype (Santibanez M, et al., 2012). A German uranium miner cohort study however found a positive statistically non-significant relationship between stomach cancer mortality and occupational exposure to arsenic dust, fine dust, and absorbed dose from α and low-linear energy transfer radiation (Kreuzer M, et al., 2012).

**Blood Group-A**

An association between gastric carcinomas and the blood group A has been reported (Aird I, et al., 1953; Haenszel W, et al., 1976), which may be related to the interaction between the Lewis blood group antigen and H. pylori (Carneiro F, et al., 1996). The association of the blood group A with males, with diffuse-type gastric cancer is stronger than with females, or intestinal-type gastric cancer (Kramer B S, et al., 1995).
Gene polymorphism

In recent years, genetic polymorphisms have come to be recognized as crucial factors determining disease susceptibility. Host gene polymorphisms frequently influence the magnitude of the host response, and this interindividual variation contributes to the clinical outcome. The development of gastric cancer in a milieu of chronic inflammation induced by *H. pylori* may be significantly influenced by host gene polymorphisms.

The proinflammatory cytokine interleukin-1 (IL-1) gene cluster containing *IL-1β* and *IL-1RN* encodes IL-1β and the IL-1β receptor antagonist, respectively, and the risk of gastric cancer and its precursor lesions is increased in the presence of *H. pylori* by polymorphisms in these genes. An increased risk of developing *H. pylori*-mediated hypochlorhydria and gastric atrophy is associated with the *IL-1B*-31*C* or -511*T, and the *IL-1RN*2/*2 genotypes (El-Omar E M, et al., 2000). These genotypes are also associated with a 2-3 fold increase in the risk of developing gastric cancer, compared to individuals with less proinflammatory genotypes (El-Omar E M, et al., 2000, 2003; Figueiredo C, et al., 2002). Although a range of studies have reported lower risks, recent meta-analyses have supported the findings of the higher risk of the *IL-1B*-511*T, and the *IL-1RN*2 genotypes, particularly in association with ethnic group and tumour type (Camargo M C, et al., 2006; Kamangar F, et al., 2006; Wang P, et al., 2007).

Although IL-1 is one of the most important proinflammatory cytokines mediating the effects of *H. pylori* infection (El-Omar E M, et al., 2000),
polymorphisms in other proinflammatory cytokines, such as TNFα (308*A) and IL-10 (ATA/ATA), have also been associated with increased risk for gastric cancer (El-Omar E M, et al., 2003). The more proinflammatory genotypes an individual has, the higher the risk of developing gastric cancer (Figueiredo C et al., 2002). Gastric cancer and H. pylori infection has been linked with the human leukocyte antigen (Magnusson et al, 2001), with the *1601 allele significantly increasing the risk of gastric cancer (odds ratio 8.7; 95% CI 2.7-28). This association is seemingly independent of H. pylori infection, being stronger in H. pylori-negative patients and in diffuse-type carcinomas (Correa P, 2002).

The MUC-1 mucin is a glycoprotein involved in the protection and lubrication of epithelial surfaces, detecting potential external insults and interacting with signal transduction and cell adhesion proteins (Gendler S J, 2001). MUC-1 contains a variable number of tandem repeats, with higher numbers of repeats encoding larger proteins better able to respond to external stimuli. Gastric cancer patients exhibit higher proportions of smaller MUC-1 proteins, with smaller alleles linked to gastric atrophy and intestinal metaplasia (Carvalho F, et al., 1999; Correa P, 2002).

**Diagnosis of Gastrointestinal Cancer**

The diagnosis of cancer involves the analysis of tissue and cytology specimens obtained through several procedures, including surgical biopsy, core or aspirational needle biopsy, venipuncture, pleural or ascitic tap, scraping of tissue surfaces and collection of exfoliative cells from urine and
sputum. Conventional histopathology based on assessing morphology has remained the standard diagnostic method for many years but development of advanced sophisticated technologies like mass spectrometry microarray and automated DNA sequencing have opened new avenues in cancer diagnosis and therapeutics.

1. Clinical Symptoms

Clinical symptoms of cancer vary according to the type and nature of the cancer and its location in different organs. These include gastrointestinal obstruction which may be accompanied by bleeding which is presented as diarrhea and vomiting (commonly associated with tumors invading the stomach, small intestine, large intestine, or colon), hematuria (in tumors of the kidney or bladder), Cushing’s disease, hypoglycemia, etc. (in hormone-producing tumors such as some pancreatic, thymic and hepatic tumors), hematological disturbances (Staszewski H, et al., 997) as anemia, polycythemia, granulocytosis etc. and neurologic symptoms (Darnell R B, et al., 2006; Rees J H, et al., 2004) such as loss of coordination or seizures (in tumors of the brain or spinal cord). Cancers producing non-specific symptoms are extremely difficult to be diagnosed for their location, referred to as paraneoplastic disorders. These include weight loss, low-grade fever, seizures, lethargy, loss of appetite, diarrhea, skin rash, hair loss, and general arthritic-like symptoms. These types of cancers require specialized diagnostic techniques such as laboratory screening tests, X-rays, CT scan, MRI
etc. which can provide a means for earlier diagnosis and perhaps better long-term prognosis.

2. Imaging

A diagnosis of malignancy is frequently suspected based on imaging information, later confirmed on histology. Until now, exploratory surgery or limited radiologic evaluations are most commonly used techniques for cancer diagnosis and staging. With the advent of computed tomography (CT) and magnetic resonance imaging (MRI), it became possible to obtain important structural and anatomic information. Molecular imaging with magnetic resonance spectroscopy (MRS) and positron emission tomography (PET) is currently possible in clinical practice. These modalities permit functional, biochemical and physiologic assessment of important aspects of malignancy. Sites in which imaging plays a key role for the diagnosis include brain, breast, lung and mediastinum, the tumors arising from the abdominal organs, retroperitoneum and bones. Conventional radiography provides the easiest way to diagnose the tumors of gastrointestinal tract, lungs, brain, liver, urinary bladder, breast, bone, joints etc in the pet and domestic animals (Cole R, et al., 2007; Drost, W T, et al., 1996; Russo M, et al.,1999; Steinberg H, et al., 2006; Wuersch K, et al., 2009)

3. Ultrasound

Ultrasonography uses high frequency broadband sound waves in the megahertz range that are reflected by tissue to varying degrees to produce images (up to 3D). It used in the diagnosis of cancers of abdominal organs,
heart, breast, muscles, tendons, arteries and veins. It is useful in aiding the characterization of lesions (shape, size, and density) found on screening mammograms in women with dense breasts (Kolb T M, 2000.) and in bitches having mammary tumors (Gonzalez de Bulnes A, et al., 1998). While it may provide less anatomical detail than techniques such as CT or MRI, it has several advantages which make it ideal in numerous situations, in particular that it studies the function of moving structures in real-time, emits no ionizing radiation, and contains speckle that can be used in elastography. Ultrasound has also been used for the diagnosis of the tumourous conditions of the abdominal organs of domestic and wild animals (Ferreira V L, et al., 2010). Tumours of the hollow organs such as urinary bladder can be diagnosed easily by the ultrasonographic technique (Hoque M, et al., 2002). Singh C, et al., (2009) used ultrasound guided biopsies (USGB) and ultrasound guided fine needle aspiration biopsies (USGFNAB) to diagnose the hepatic affections in dogs and benefits of these techniques can be utilized for the diagnosis of tumours of other internal organs. High resolution ultrasound can be used to evaluate the tumour volume accurately in the murine orthotopic tumour models without sacrifice (Kraaij R, et al., 2002; Pezold J C, et al., 2006).

4. Computed Tomography

Recent innovations include spiral (helical) CT, multiphase imaging and multi detector scanning. Potential patient benefits include rapid data acquisition and improved detection and characterization of lesions. Spiral CT
currently is the preferred technique for detecting cancerous lesions in pulmonary organs and liver prior to metastasectomy and for surgical planning of pancreatic and renal cancer treatment. Cases of nasal tumours in dogs (Kondo Y, et al., 2008) and horses (Veraa S, et al., 2008) can be diagnosed and evaluated on the basis of the CT scan New roles for spiral CT include the detection of pulmonary emboli, CT angiography and endoscopic viewing of hollow organs. Dogs and cats are prone to brain and spine tumours which are life threatening and need to be diagnosed as early as possible before they attain incurable stage. Brain tumours includes the tumours of pituitary, cerebrum, cerebellum, hypothalamus etc. and tumours of spine divide as extradural, intradural - extramedullary, or intramedullary, among them 50% are extradural, whereas 35% and 15% are intradural-extramedullary and intramedullary, respectively (Narama I, et al., 1992). So it is certain that CT scan can play an important role in the diagnosis of tumours of brain and spine in dogs and cats (Bast R C, et al., 2003; Iwamoto K S et al., 1993; Mauldin G N, et al., 1990; Pollard R E, et al., 2010).

5. Magnetic Resonance Imaging

In this technique powerful magnets are used to polarize and excite hydrogen nuclei in water molecules in tissue, producing a detectable signal which is spatially encoded, resulting in images of the body. MRI has a number of imaging benefits including superb soft tissue contrast, multiplanar and 3D image acquisition, freedom from ionizing radiation and bony artifacts, and ability to acquire biological and physiological information. Recent advances
with the use of supercoils have resulted in an increase in sensitivity and specificity (Kaiser W A, et al., 1992). Contrast agents used in it are chelates of gadolinium, a lanthanide with three unpaired electrons, which has a very strong magnetic field. MRI is the imaging technique of choice for evaluating tumors in brain, head and neck, spine, breast (when mammography is technically difficult owing to dense breast, silicone implants and scarring due to surgery/ trauma), liver and adrenal glands. Recent advances include increased speed of data acquisition and the ability to visualize function superimposed on anatomical changes. Breast MRI has been shown to be capable of detecting early breast cancer, with sensitivities in the range of 95–100%, i.e. a low false negative rate (Harms S, et al., 1999; Orel S G, et al., 1994). In veterinary field MRI has been used frequently in detecting macrotumours of brain and spine in dogs (Duesberg C A, et al., 1995; Kippenes H, et al., 1999). Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is a newer technique by which one can evaluate the kinetic parameters such as blood flow, perfusion, vascular permeability, and the fraction of interstitial space within a tumor. These parameters derived from DCE-MRI present information which are appropriate to noninvasively differentiate canine brain tumors (Zhao Q, et al., 2010).

6. Metabolic and Functional Imaging

Functional imaging is a recent tool used in oncology and its uses includes characterization of indeterminate lesions on conventional imaging, cancer staging and monitoring response to treatment.
7. Positron emission tomography (PET)

PET can detect biologic changes in vivo using radiolabeled tracers. It represents the metabolic activity of underlying tissue processes such as glucose, oxygen and amino acid metabolism or measures receptor density status. Fluorine-18 fluorodeoxyglucose (18FFDG) is used most commonly, and closely mimics endogenous molecules (Grahek D, et al., 2004; Kaiser W A et al., 1992). FDG enters cells and is phosphorylated to FDG-6-phosphate, which becomes trapped within malignant tumor cells with high glucose metabolism. PET is the most accurate non-invasive technique for detecting and staging lung cancer. It is superior to CT arterial portography in detecting intrahepatic metastases in colorectal cancers and can identify metastatic deposits in lymph nodes that are still <1 cm in size and considered benign by CT. In contrast, PET may recognize large masses, such as post therapy fibrotic tissue, as benign if minimal FDG uptake is demonstrated. Limitation with PET to tumor detection is that increased FDG uptake can also be demonstrated in inflammatory tissue.

8. Magnetic resonance spectroscopy

MRS is a non-invasive method for studying tumor biochemistry and physiology. It measures signals from chemical compounds within tissues; P31 MRS provides information on tissue energetic and pH while H1- MRS conveys information on cell membrane synthesis and degradation, reflecting cellular proliferation and necrosis. MRS resonances can provide diagnostic information on tumor grade and are used to monitor tumor response to
therapy. In a review by Katz-Brull R, et al., (2002) in a study of five breast H-MRS performed in four independent centers around the world to date, the combined analysis of the data from a total of 153 tumors demonstrated sensitivity and specificity as high as 92% in distinguishing malignant from benign tumors using the choline signal. Of special interest, in a subgroup of 20 younger women the sensitivity and specificity of the method approached 100% (Katz-Brull R, et al., 2002).

9. Cytologic and Histopathological Technique

Histopathology is still a gold standard for diagnosis of tumors but it alone does not provide sufficient details of the cellular changes which could predict the clinical behaviour of the tumor. Even then histopathological examination of the tumor cells by any expert oncologist can give an accurate diagnosis about the type of tumor and possible malignancy status. Serous effusions from pleural, peritoneal or pelvic cavity can act as biopsy material for diagnosis of tumors of those regions as these may consist the cancerous cells. Oyafuso M S, et al., (1996) compare the results of cytological diagnosis of tumors in Brazil, and found that sensitivity, specificity, efficiency, as well as positive and negative predictive values for cytologic diagnosis were 44.5%, 95.7%, 50.1%, 98.7% and 20%, respectively. Presence of hyperchromatic nuclei, more nucleus to cytoplasm ratio, disorientation of the cells, etc. are the cellular changes observed in cancer cell. Presences of mitotic figures in the cells are also related with neoplastic change in the tissues. During studies on the canine mammary and skin tumors it was
found that number of mitotic figures in the malignant tumours was significantly higher than the benign tumours (Pawan K, et al., 2010; Reddy G, et al., 2009). Special staining procedures can differentiate the different types of tumours and thus help in diagnosis such as toluidine blue stain differentiate mast cell tumour from other tumours as it stains the metachromatic granules present in mast cells.

10. Serological Methods

Serological methods used in estimation of serum tumor markers are ELISA and RIA (Schrohl A S, et al., 2003, Wu J T, et al., 2007). The ELISA is typically used to detect and quantify antigen within biological fluids, in which the Dual-Antibody Sandwich ELISA is being used for measuring the concentration of 80% of tumor markers in blood or serum. RIA is one of the most sensitive technique for detecting antigen or antibody. The principle involves competitive binding of radiolabelled antigen and unlabelled antigen to a high-affinity antibody. Gamma emitting isotope such as Iodine and beta emitting isotope such as tritium are also routinely used as labels. The important step in the RIA is the determination of the amount of antibody needed to bind 60% - 70% of a fixed quantity of radioactive antigen. Determination of amount of bound labeled antigen can be done by precipitating the Ag- Ab complex to separate it from free antigen and the radio activity in the precipitate can be measured (Chan D W, et al., 1997). The presence of CEA, AFP, PSA and other markers in the serum of the cancer patients can be detected with the help of ELFA (Enzyme linked florescent
assay). The test measures the amount of CEA that may appear in the blood of some people who have certain kinds of cancers, especially large intestine (colon and rectal) and breast cancer. It may also be present in people with cancer of the pancreas, ovary, or lung (Blast R C, 2003; Thriveni K, et al., 2007).

11. Fluorescence in Situ Hybridization (FISH) Technique

This technique involved the specific hybridization of a labeled nucleic acid probe to complementary gene sequence and subsequent visualization by autoradiographic or immunocytochemical method in tissue section, smears or cytocentrifuged cell suspensions. Chromosome abnormalities are frequently found in malignant cells. Chromosome rearrangements can be duplications (addition of chromosome), deletions (loss of whole or parts of chromosomes), segmental amplifications (random reiteration of segments or extra fragments), translocations (exchange between chromosomes) and inversions (reversal of orientation). It is applicable to interphase cells and is more sensitive compared to conventional cytogenetic. Comparative genomic hybridization (CGH) is a newly described method developed in 1992 and used globally for studies of chromosomal gains and losses in genomic complement. In CGH, test and reference genomic DNA are first differentially labeled with different fluorescent dyes and co hybridized to normal metaphase chromosomes. Then, fluorescent signals along each chromosome are examined and analyzed to provide a cytogenetic pattern of gains and losses. Several investigators have found this method to be useful in cancer
studies, suggesting that different tumor types or different stages of tumor progression have distinct CGH patterns (Forozan F, et al., 1997). These quantitative changes are related to modification in expression level of genes located in the target region. They found a substantial degree of correlation between the two levels of information. To increase resolution, several groups have adapted array technology to CGH, leading to so-called array-CGH (Pollack J R, et al., 1999). Array-CGH has now been established as a new method for molecular characterization of cancers. Moreover, it can serve as a starting point for further screening investigations, such as genome-wide gene expression profiling.

12. Polymerase Chain Reaction (PCR)

Molecular oncology studies the alterations in genetic and biochemical processes at the molecular level. It helps in establishing a definitive diagnosis and classification of tumors based on the recognition of complex profiles ('finger-prints') or unique molecular alteration that occur in specific tumor types. The changes can be studied on chromosomes, DNA or RNA. Microsatellite markers, also known as simple sequence repeats or SSRs (Litt M, et al., 1989) are scattered widely within the biological genomes and closely linked with many important genes. In carcinogenesis, microsatellites often display loss of heterozygosity (LOH) as tumour suppressor genes. These are highly polymorphic repetitive DNA sequences that are randomly distributed throughout eukaryotic genomes displaying high levels of variation and are having high mutation rates (1-4 per generation) (Weber J et
The PCR process was originally developed to amplify short segments of a longer DNA molecule (Robertson L E, et al., 1992). PCR allows early diagnosis of malignant diseases such as leukemia and lymphomas, which is currently the highest developed in cancer research and is already being used routinely. PCR assays can be performed directly on genomic DNA samples to detect translocation-specific malignant cells at a sensitivity which is at least 10,000 fold higher than other methods. Quantitative PCR methods allow the estimation of the amount of a given sequence present in a sample a technique often applied to quantitatively determine levels of gene expression. Real-time PCR is an established tool for DNA quantification that measures the accumulation of DNA product after each round of PCR amplification. Mackay et al. (Martins A, et al., 2010) opined that Real-time quantitative PCR is a very powerful and accurate technique to examine expression patterns of different oncogene, suppressor genes in different cancerous conditions. Real-time PCR has engendered wider acceptance of the PCR due to its improved rapidity, sensitivity, reproducibility and the reduced risk of carry-over contamination. There are currently five main chemistries used for the detection of PCR product during real-time PCR. PCR act as an important tool for the diagnosis of the virus induced tumours of the animals such as cutaneous papillomatosis in cattle, urinary bladder tumours of cattle (De Villiers E M, et al., 2004; Pawaiya R, et al., 2005) and buffaloes (Sylvestre O, et al., 2009), equine sarcomas (Nasir L, et al., 2008), papillomatosis in dogs (Goldschmidt M N, et al., 1998; Narama I, et al., 1992) etc.
13. Microarray

Microarray has emerged as a powerful tool to increase the potential of standard methods through genome wide biology studies. DNA microarray technology is a promising approach that allows both qualitative and quantitative screening for sequence variations in the genomic DNA of cancer cells. DNA microarray-based sequence analysis uses comparative hybridization to obtain information ranging from mutational detection to polymorphism genotyping. Sequencing by hybridization is conceptually based on the construction of unknown sequences from hybridization data (Wallraff G, et al., 1997). Labeled DNA for analysis binds strongly only to those targets that are fully complementary to one of its subsequences. Specific binding profile is further checked by a computational algorithm to deduce the whole original sequence.

14. Mutational Analysis

Detection of mutations in cancer is of major importance for both basic understanding of the disease process and clinical practice. High-density oligonucleotide arrays are commonly used to achieve this purpose. Many early applications of this method concerned breast cancer-associated genes BRCA1 and BRCA2 (Favis R et al., 2000; Hacia J G, 1996, 2000). From this initial success, one can easily predict the impact of specific “mutation arrays,” which test for a variety of known mutations in numerous oncogenes, tumor suppressors, and other genes shown to be of interest in cancer.
15. Polymorphism Genotyping

Microarray is an appropriate tool to understand how sequence polymorphism may impact biologic functions and be associated with heritable phenotypes. Single nucleotide polymorphisms (SNPs) are the most abundant form of DNA polymorphisms. SNP microarray is an oligoarray in which SNPs are screened by a set of oligonucleotide probes. In a first approach, different oligonucleotides can be used to identify several thousand SNPs and then specific oligonucleotides can be used to genotype these SNPs in various samples (Sapolsky R J, et al., 1999). SNP microarrays have potential applications in loss of heterozygosity (LOH) analysis, in disease susceptibility and pharmaco- and toxicogenetic studies.

Characteristics of Free radicals and Oxidants

Free radicals are defined as atoms or molecules containing one or more unpaired electrons which are highly unstable and reactive substances. The most important ROS are the superoxide anion radical $O_2^-$, hydrogen peroxide ($H_2O_2$), alkoxyl (RO·), peroxyl (ROO·), hydroxyl radicals (·OH), and hypochlorous acid (HOCl). Other non-oxygen species exist as reactive nitrogen species (RNS), such as nitric oxide (NO·) and peroxynitrite having important bioactivity. ROS is continuously generated in physiological conditions but are effectively eliminated by intracellular and extracellular antioxidant systems (Halliwell B, et al., 1999).
Sources of Reactive Oxygen Species

ROS are produced from molecular oxygen as a result of normal cellular metabolism. ROS can be divided into 2 groups: free radicals and nonradicals. Molecules containing one or more unpaired electrons and thus giving reactivity to the molecule are called free radicals. When 2 free radicals share their unpaired electrons, non-radical forms are created. The 3 major ROS that are of physiological significance are superoxide anion (O$_2^-$), hydroxyl radical (OH$^-$), and hydrogen peroxide (H$_2$O$_2$).

*Endogenous and exogenous sources of reactive oxygen species (ROS)*
Major Reactive Oxygen Species (ROS)

<table>
<thead>
<tr>
<th>Radical</th>
<th>Non radical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide</td>
<td>O$_2^*$</td>
</tr>
<tr>
<td>Hydroxyl radical</td>
<td>OH*</td>
</tr>
<tr>
<td>Organic radical</td>
<td>R*</td>
</tr>
<tr>
<td>Peroxyl radical</td>
<td>Roo*</td>
</tr>
<tr>
<td>Alkoxyl radical</td>
<td>Ro*</td>
</tr>
<tr>
<td>Thiyl radical</td>
<td>RS*</td>
</tr>
<tr>
<td>Sulphonyl radical</td>
<td>ROS*</td>
</tr>
<tr>
<td>Thiyl peroxyl radical</td>
<td>RSOO*</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>H$_2$O$_2$</td>
</tr>
<tr>
<td>Ozone</td>
<td>O$_3$</td>
</tr>
<tr>
<td>Organic hydrogen peroxide</td>
<td></td>
</tr>
<tr>
<td>Hypochlorous acid</td>
<td>HOCl</td>
</tr>
<tr>
<td>Singlet Oxygen</td>
<td>$^{1}$O$_2$</td>
</tr>
</tbody>
</table>

Superoxide anion radical and hydroxyl radical

Superoxide anion is formed by the addition of one electron to the molecular oxygen. (Miller D M, et al., 1990) This process is mediated by nicotine adenine dinucleotide phosphate [NAD(P)H] oxidase or xanthine oxidase or by mitochondrial electron transport system. The major site for producing superoxide anion is the mitochondria, the machinery of the cell to produce adenosine triphosphate. Normally, electrons are transferred through mitochondrial electron transport chain for reduction of oxygen to water, but approximately 1 to 3% of all electrons leak from the system and produce superoxide. NAD(P)H oxidase is found in polymorphonuclear leukocytes, monocytes, and macrophages. Upon phagocytosis, these cells produce a burst of superoxide that lead to bactericidal activity. Superoxide is converted into hydrogen peroxide by the action of superoxide dismutases (SODs, EC 1.15.1.1). Hydrogen peroxide easily diffuses across the plasma membrane.
Hydrogen peroxide is also produced by xanthine oxidase, amino acid oxidase, and NAD(P)H oxidase (Dupuy C, et al., 1991; Granger D N, et al., 1991) and in peroxisomes by consumption of molecular oxygen in metabolic reactions.

In a succession of reactions called Haber–Weiss and Fenton reactions, H₂O₂ can breakdown to OH⁻ in the presence of transmission metals like Fe²⁺ or Cu²⁺. (Fenton H, et al., 1984)

\[ \text{Fe}^{3+} + \cdot \text{O}_2 + \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + \text{O}_2 \]  
Haber-Weiss

\[ \text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \cdot \text{OH} \]  
Fenton reaction

O₂⁻ itself can also react with H₂O₂ and generate OH⁻ (Haber F, et al., 1934; Liochev S I, et al., 2002). Hydroxyl radical is the most reactive of ROS and can damage proteins, lipids, and carbohydrates and DNA. It can also start lipid peroxidation by taking an electron from polyunsaturated fatty acids.

**Hydrogen peroxide**

Hydrogen peroxide can be generated through a dismutation reaction from superoxide anion by superoxide dismutase. Enzymes such as amino acid oxidase and xanthine oxidase also produce hydrogen peroxide from superoxide anion. Hydrogen peroxide is highly diffusible and crosses the plasma membrane easily. Hydrogen peroxide is the least reactive molecule among reactive oxygen species and is stable under physiological pH and temperature in the absence of metal ions. Hydrogen peroxide is a weak oxidizing and reducing agent and is thus regarded as being poorly reactive. Hydrogen peroxide can generate the hydroxyl radical in the presence of metal ions and superoxide anion \((\cdot \text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \cdot \text{OH} + \text{OH}^- + \text{O}_2)\) (Halliwell B,
Hydrogen peroxide can produce singlet oxygen through reaction with superoxide anion or with HOCl or chloroamines in living systems. Hydrogen peroxide can degrade certain heme proteins, such as hemoglobin, to release iron ions.

**Figure: Sources, generation of reactive oxygen species and the defense mechanisms against damage by active oxygen species in human disease. (Jose et al, 1999).**

**Hypochlorous acid**

Granulocytic enzymes further expand the reactivity of $H_2O_2$ via eosinophil peroxidase and myeloperoxidase (MPO). In the presence of chloride ion, $H_2O_2$ is converted to hypochlorous acid (HOCl). HOCl is highly oxidative and plays an important role in killing of the pathogens in the airways. (Klebanoff S J, et al., 2005) However, HOCl can also react with DNA and induce DNA–protein interactions and produce pyrimidine oxidation products and add chloride to DNA bases. (Whiteman M, et al., 1997; Kulcharyk P A, et al., 2001) Eosinophil peroxidase and MPO also contribute to the oxidative stress by modification of proteins by halogenations, nitration, and protein cross-links via tyrosyl
Peroxyl radicals

Other oxygen-derived free radicals are the peroxyl radicals (ROO\(^{-}\)). Simplest form of these radicals is hydro-peroxyl radical (HOO\(^{-}\)) and has a role in fatty acid peroxidation. Free radicals can trigger lipid peroxidation chain reactions by abstracting a hydrogen atom from a side chain methylene carbon. The lipid radical then reacts with oxygen to produce peroxyl radical. Peroxyl radical initiates a chain reaction and transforms polyunsaturated fatty acids into lipid hydroperoxides. Lipid hydroperoxides are very unstable and easily decompose to secondary products, such as aldehydes (such as 4-hydroxy-2,3-nonenal) and malondialdehyde (MDAs). Isoprostanes are another group of lipid peroxidation products that are generated via the peroxidation of arachidonic acid and have also been found to be elevated in plasma and breath condensates of asthmatics. (Wood L G, et al., 2000; Montuschi P, et al., 1999) Peroxidation of lipids disturbs the integrity of cell membranes and leads to rearrangement of membrane structure.

Antioxidants

An Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants (enzymatic & non-enzymatic) terminate these chain reactions by removing radical...
intermediates and inhibiting other oxidation reactions by being oxidized themselves. So, antioxidants are often reducing agents such as thiols or polyphenols (Duarte T L, et al., 2005). Although oxidation reactions are crucial for life, they can also be damaging; plants and animals contain various antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and peroxidases. Low levels of antioxidants or inhibition of the antioxidant enzymes causes oxidative stress and may damage or kill cells (Valko M, et al., 2007).

The antioxidant defense systems function through blocking the initial production of free radicals, scavenging the oxidants, converting the oxidants to less toxic compounds, blocking the secondary production of toxic metabolites or inflammatory mediators, blocking the chain propagation of the secondary oxidants, repairing the molecular injury induced by free radicals or enhancing the endogenous antioxidant defense system of the target. These defense mechanisms act cooperatively to protect the body from oxidative stress. The antioxidant defense system consists of powerful enzymatic and non-enzymatic antioxidants (Halliwell B. 2007)

<table>
<thead>
<tr>
<th>Enzymatic and Non-enzymatic antioxidants</th>
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<tbody>
<tr>
<td><strong>Enzymatic</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>Catalase</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
</tr>
<tr>
<td>Glutathione-S-transferase</td>
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</table>
All cells in the body contain powerful antioxidant enzymes. The three major classes of antioxidant enzymes are the superoxide dismutases, catalases and glutathione (GSH) peroxidases. In addition, there are numerous specialized antioxidant enzymes reacting with and detoxifying oxidants (Valko M, et al., 2007)

**Superoxide dismutases (SOD)**

They are a class of closely related enzymes that catalyse the breakdown of the superoxide anion into oxygen and hydrogen peroxide (Zelko I, et al., 2002). They are present in almost all aerobic cells and in the extracellular fluids. They contain metal ions that can be copper, zinc, manganese or iron. In humans, the copper/zinc superoxide dismutase is present in the cytosol, while manganese superoxide dismutase is present in the mitochondria. There also exists a third form of superoxide dismutase in extracellular fluids, which contains copper and zinc in its active sites (Johnson F, 2005). Superoxide dismutase removes O2. – by catalyzing a dismutation reaction. In the absence of superoxide dismutase, this reaction
occurs non-enzymatically but at a very slow rate (Nozik-Grayck E, et al., 2005)

**Catalase**

Catalase (H$_2$O$_2$ oxidoreductase) is a tetramer of four polypeptide chains, each over 500 amino acids long, contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide. Catalase can decompose hydrogen peroxide (H$_2$O$_2$) in reactions catalyzed by two different modes of enzymatic activity: the catalatic mode of activity (2 H$_2$O$_2$ → O$_2$ + 2 H$_2$O) and the peroxidatic mode of activity (H$_2$O$_2$ + A H$_2$ → A + 2 H$_2$O). Catalase has one of the highest turnover rates of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second. Decomposition of H$_2$O$_2$ by the catalytic activity of catalase follows the fashion of a first-order reaction and its rate is dependent on the concentration of H$_2$O$_2$ (Valko M, et al., 2007; Berg J M, et al., 2002).

Catalase is an unusual enzyme since, although hydrogen peroxide is its only substrate, it follows a ping-pong mechanism. Here, its cofactor is oxidised by one molecule of hydrogen peroxide and then regenerated by transferring the bound oxygen to a second molecule of substrate (Kabel A M, et al., 2013)

Catalase is present in all prokaryotes and eukaryotes. With the exception of erythrocytes, it is predominantly located in peroxisomes of all types of mammalian cells where H$_2$O$_2$ is generated by various oxidases. Since H$_2$O$_2$ serves as a substrate for certain reaction that generate the highly
reactive hydroxyl radical, catalase is believed to play a role in cellular antioxidant defense mechanisms by limiting the accumulation of H₂O₂ (Ho YS, et al., 2004)

The role of catalase in defending cells and tissues against oxidative stress has been studied extensively. Over expression of catalase renders cells more resistant to toxicity of H₂O₂ and oxidant-mediated injury. In addition, transgenic mice overexpressing catalase are protected against myocardial injury following administration of adriamycin and development of hypertension from treatment with norepinephrine or angiotensin. Catalase-deficient patients are phenotypically normal with the exception of an increased tendency to development of progressive oral gangrene as a result of tissue damage from H₂O₂ produced by peroxide-generating bacteria such as streptococci and pneumococci as well as by the phagocytic cells at the sites of bacterial infection (Yang H, et al., 2003)

**Glutathione**

The glutathione system includes glutathione, glutathione reductase (GR), glutathione peroxidases (GPx) and glutathione S-transferases. Glutathione peroxidase is an enzyme that catalyzes the breakdown of hydrogen peroxide and organic hydroperoxides. Glutathione S-transferases are another class of glutathione-dependent antioxidant enzymes that show high activity with lipid peroxides (Sharma R, et al., 2004) These enzymes are at high levels in the liver and also help in detoxification metabolism (Hayes J, et al., 2005).
Glutathione reductase (GR) is a crucial enzyme that reduces glutathione disulfide (GSSG) to the sulfhydryl form (GSH) by the NADPH-dependent mechanism, an important cellular antioxidant system. Due to its significance, the enzyme has been purified from a number of animals, plants and microbial sources and studied in an effort to identify and explain its structure, kinetic mechanism and molecular properties (Linster C L, et al., 2007). Its kinetic mechanism is known to be a ping-pong/sequential ordered model. GR is a flavoprotein that contains two FAD molecules as a prosthetic group, which is reducible by NADPH. GR is one of the thermostable enzymes. GR belongs to the defense system protecting the organism against chemical and oxidative stress. Deficiency of GR is characterized by hemolysis due to increased sensitivity of erythrocyte membranes to H\textsubscript{2}O\textsubscript{2} and contributes to oxidative stress which plays a key role in the pathogenesis of many diseases (Ulusu N N, et al., 2007)

**Non-Enzymatic antioxidants**

**Ascorbic acid**

Ascorbic acid or vitamin C is a monosaccharide antioxidant found in both animals and plants but cannot be synthesised in humans and must be obtained from the diet. In cells, it is maintained in its reduced form by reaction with glutathione. Ascorbic acid is a reducing agent that can reduce and thereby neutralize reactive oxygen species such as hydrogen peroxide (Linster C L, et al., 2007).
Vitamin E

Vitamin E (α-tocopherol) is the most important lipid-soluble antioxidant and protects cell membranes against oxidation by reacting with the lipid radicals produced in the lipid peroxidation chain reaction and removing the free radical intermediates. Tocotrienols may have a specialised role in neuroprotection (Sen C, et al., 2006)

Carotenoids

Carotenoids are compounds with lipophilic properties that have antioxidant functions in lipid phases. Beta-carotene besides being a precursor to vitamin A has potent antioxidant properties as it removes singlet oxygen thus protects against free radical attack. They are present in liver, egg yolk, milk, butter, spinach, carrots, tomato and grains (Linster C L, et al., 2007).

![Fig. - Mutual association between oxidants and antioxidants.](image)

Oxidative stress

Oxidative stress can be defined as any disturbance in the balance of antioxidants and pro-oxidants in favor of the later due to different factors such as aging, drug actions and toxicity, inflammation and/or addiction (Sies H, 1985). It is in general, excess formation or/and insufficient removal of highly reactive molecules such as reactive nitrogen species (RNS) and reactive oxygen species (ROS) (Johansen et al., 2005). Oxygen is highly reactive specie that has the ability to become part of potentially harmful and damaging molecules (Free Radicals). Oxidative stress causes healthy cells of the body to lose their function and structure by attacking them. Up until now, pathogenesis of about more than 50 diseases has been implicated by free radicals. It is when the antioxidant level is limited that this damage can become debilitating and cumulative (Mark P, 1996). Damage to DNA, proteins, and other macromolecules due to oxidation has been implicated in the pathogenesis of a wide variety of diseases, most notably cancer and heart disease (Halliwell B, 1994).

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<th>Oxidative stress induced organ damage</th>
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### Oxidative stress and gastrointestinal diseases

Oxidative stress plays important roles in pathogenesis of gastro-intestinal diseases, which include mucosal damage, gastro-intestinal ulcers, and cancer. Although gastric ulcer can be generated by different factors, *e.g.*, non-steroidal anti-inflammatory drugs (NSAIDs), thermal stress, ethanol, and *H. pylori* infection, leading to oxidative damage through free radical generation (specially OH⁻) and subsequent apoptotic responses of gastric mucosa (Ding S Z, et al., 2007). In addition, gastro-intestinal diseases are associated to increased oxidative stress and oxidants levels, such as glutathione, lipid peroxidation, myeloperoxidases, protein carbonyl, etc (Ganguly K, et al., 2012). Furthermore, pathogens are directly involved in aggravating oxidative stress; for example, complete eradication of *H. pylori* is reported to attenuate oxidative stress in gastric mucosa (Banerjee A, et al., 2013).

Although certain types of gastro-intestinal inflammations, like ulcerative colitis, hepatitis, *H. pylori* infection, are more prone to develop cancer, the reasons are still not well elucidated. Inflammation and subsequent elevated oxidative stress might be the factors for aggravating...
chronic inflammation and inducing malignant transformation. Transgenic mice expressing hepatitis B protein in liver develop chronic hepatitis with elevated levels of 8-oxo-dG, leading to hepatocellular carcinoma (Hagen TM et al., 1994). It is well accepted that inflammation is always accompanied with elevated oxidative stress in cancer. Gastric cancer patients (with normal renal and hepatic functions) are found to have significantly increased lipid peroxidation levels (Reddy E P, et al., 2010). Gastric carcinoma patients have significantly higher myeloperoxidase activity than controls, both before and after operation, although total antioxidant status was decreased post-operation (Czygier M, et al., 2010). Gastric cancers are also associated with augmented protein oxidation, although no differences are found in oxidative stress parameters and antioxidant enzyme activities between anti-H. Pylori IgG positive and negative gastric cancer patients (Noyan T, et al., 2009).

**Gastric enzyme - Pepsinogen**

Pepsinogen is an inactive form of pepsin, which is the most important proteolytic enzyme of gastric juice. (Tanaka Y, et al., 1991). Pepsinogen is synthesised in the gastric mucosa by the chief and neck cells of the glandular ducts and is secreted into the lumen of the gastric gland (Simpson L, et al., 1980). Human pepsinogen can be separated on polyacrylamide gel electrophoresis into seven isozymogens, consisting of two immunochemically distinct groups: pepsinogen A (pepsinogen-I) and pepsinogen C (pepsinogen-II). In contrast to pepsinogen C, pepsinogen A is secreted in the urine and shows considerable interindividual heterogeneity. Several genetic models
have been proposed to explain the inheritance of urinary pepsinogen A (Korsnes L, et al., 1980). Increased activation of pepsinogen into pepsin by enhanced acidity of gastric contents can cause ulcers in humans and animals (Tanaka Y, et al., 1991; Saez-Alquezar A, et al., 1978; Vianello F, et al., 1988).

**Pepsinogen-C/ Pepsinogen-II**

Progastriscin/ Pepsinogen-C/ pepsinogen-II/ PGC, acts as a proteolytic enzyme of gastric secretion (EC 3.4.23.3). It is a 40-kDa zymogen, an inactive form of aspartic protease (Foltmann B, et al., 1982). PGC imparts aspartyl proteinaselike features and is mainly synthesized in the gastric mucosa. After synthesis, it is secreted into the gastric lumen where the proenzyme is converted into its active proteolytic form under certain acidic conditions (Moore S A, et al., 1995). It is widely distributed in the gastrointestinal tract of various species and constitutes a major proteolytic enzyme present in the gastric fluid (Samloff I M, 1989). The primary structure deduced from its c-DNA and genomic clones reveals that PGC is a single polypeptide chain of 388 amino-acid residues and shares a high degree of sequence identity to that of other aspartyl proteinases, e.g. pepsinogen A, procathepsin D, procathepsin E and prorenin (Hayano T, et al., 1988; Taggart R T, et al., 1989). Another biochemical study shows that the conversion of human progastricsin to gastricsin is accompanied by two intermediates (Foltmann B, et al., 1982). The first intermediate species has formed rapidly after a sudden drop in Ph, 4.0. Recently, the second intermediate has been characterized structurally (Khan A R, et al., 1997).
Role of PGC in the gastric tumor progression

Gastric adenocarcinoma is most common disease in Asian countries and common cause of mortality in Europe (Pinto-Correia A L, et al., 2006). It is well documented that any change in PGC could reflect the degree of gastric disease and differentiation (Tsukamoto T, et al., 2007). It is evident from epidemiological studies that Helicobacter pylori carriers have a significantly greater risk of developing gastric cancer (Zhang L, et al., 1996). It was found that PGC is a mature marker of stomach cells, and change in its expression could reflect the degree of gastric lesions. Recently, the expression of PGC in different gastric diseases was studied (Ning P F., et al., 2005), and the results showed that PGC expression was 100% in normal gastric mucosa and only 2.4% in the gastric cancer. Moreover, such expression profile of PGC decreased gradually from benign lesions to precancerous lesions to gastric cancer.

The presence of PGC in metastasis is significantly correlated with a primary tumor of the stomach, prostate, and pancreas (Duffy M J, 1996). The overexpression of PGC has also been determined in the cancerous cells, basal cell, and squamous cell carcinomas of the eyelids (Alvarez M L, et al., 2004). Similarly, extra-digestive expression of PGC has been reported in several tumors such as cervical, endometrial and ovarian (Serra D, et al., 1999), prostate (Konishi N, et al., 1999), gastric (Fernandez R, et al., 2000), stomach (Tatematsu M, et al., 1990), gall bladder (Tatematsu M et al., 1988), breast carcinomas (Serra D, et al., 1999), and cutaneous malignant melanomas.
(Quintela I, et al., 2001). Interestingly, these expressions are significantly associated with a longer overall survival of patients with breast carcinomas (Merino A M, et al., 2000). Zhang X H, et al., (2006) observed that the serum PGC-level abnormality was accompanied with a higher risk of precancerous lesions. The presence of PGC in cancer cells implicated some mature secreting function in them. However, the decrease of PGC expression indicated dedifferentiation or malignancy of cancer cells, and it was also closely related to prognosis and metastasis (Nakamura T, et al., 1997).

**PGC Gene structure and regulation**

The gene sequence of PGC from human was determined by many groups independently (Evers M P, et al., 1989; Ishihara T, et al., 1989; Takahashi K, 1992). Chromosomal localization was assigned to the PGC gene, and subsequently its evolutionary mechanism has been established (Kageyama, T, 2002). Hormonal regulation of PGC in breast cancer cells suggests that androgens, glucocorticoids, and progesterone are capable of inducing the expression of PGC gene in different breast cancer cell lines (Balbin M, 1996). The PGC gene was cloned and expressed from various species, e.g. human (Boudi F H, et al., 1990), rabbit (Kageyama T, et al., 1990), rat (Ishihara T, et al., 1989), chicken (Hayashi K, et al., 1988), and Epinephelus coioides (Feng S, et al., 2008). PGC gene was extensively characterized, and it was found that it consists of 9 exons and 8 introns (Takahashi k, 1992). The polypeptide sequence has been divided into two parts, one 59-residue-long prosegment and another 329-residue-long pepsin
moiety. Interestingly, the proposed substrate-binding sites were well conserved among fish. The gene of human PGC is located on chromosome 6 and there is only a single copy in the human genome project sequence data. Furthermore, it is suggested that PGC genes are derived from a common pepsin-like ancestral gene (Kageyama T, 2002).

Although gene expression of PGC depends on various factors, hormonal regulation is quite important among them (Ichinose M, et al., 1988). Sakamoto N, et al. (2000) found that there are four GATA and one Sox-binding motifs in 1.1 kb of the 5’ flanking region of the PGC gene, which are essential to the organ-specific expression of the PGC. Hormonal regulation of human PGC gene in the breast cancer cells is well known for a long time (Balbin M, 1996). It was observed that a special factor regulates the transcription of PGC. This factor is a 25-kDa protein that potentially binds to a specific site in the 5’ flanking region of the gene only in the presence of Mg2+ ion (Ishihara T, et al., 1989).

Direct actions of hormones on PGC genes are unknown. As suggested by the fact that mesenchyme is necessary for enzyme induction, glucocorticoids may act indirectly on PGC genes through other factors (Tsukada S, et al., 1998). In the other study, it was suggested that glucocorticoids presumably inhibit the proliferation of mucosal cells and in turn enhance the expression of pepsinogen gene (Tsukada S, et al., 1994). Recently, the amino-acid sequences of three PGCs (PGC1, PGC2 and PGC3) were deduced by cloning and nucleotide sequencing of Pacific bluefin tuna.
(Thunnus orientalis) (Tanji M, et al., 2009). It was found that all these forms contained 16 signal sequences, an activation pro-peptide and mature peptide of 321, 323, and 332 residues long in PGC1, PGC2, and PGC3, respectively.

(A) Three-dimensional structure of human PGC. The catalytic Asp residues are (light green) and cysteine residues (yellow) are shown in ball and stick model. (B). Superimposition of human PGC (green) with procine pepsin C (sky blue). The catalytic Asp residues of human PGC and procine pepsin C are shown in green and yellow, respectively. Structure was drawn from the coordinates of PDB id: 1HTR and 2PSG (Moore et al., 1995)