PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 10 (10): 919-924, 2011
ISSN 1680-5194

Relationship Between Serum Zinc, Iron and Copper Level and Apoptosis in Human Gastric Mucosa: A Cross-Sectional Study

Beitullah Alipour[1], Aida Ghaffari[1], Alireza Ostadrahimi[1],
Abdolrasoul Safaiyan[2], Jabiz Modaresi[1] and Elnaz Vaghef Mehrabany[1]
[1]Department of Nutrition, [2]Department of Vital Statistics and Epidemiology,
Faculty of Health and Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran

Abstract: Gastric cancer is the fourth most common malignancy and the second leading cause of mortality among cancer patients in the world. Nutritional and epidemiological studies have indicated that zinc, iron and copper status modulates the risk of developing cancer and on the other hand, apoptosis has been reported to play a decisive role in precancerous changes. The aim of this cross-sectional study was to investigate whether there is any relationship between serum zinc, iron and copper levels and apoptosis (as an early indicator of gastric cancer changes) in human gastric mucosa. This cross-sectional study was conducted on 62 subjects with over 18 years of age, referred to 2 hospitals (Shahid Madani and Imam Reza hospitals) in Tabriz, Iran between October and December 2008 to undergo an upper gastrointestinal endoscopy. Serum levels of zinc, iron and copper were measured by atomic absorption spectroscopy and apoptosis was detected by TUNEL technique. Stepwise regression was exploited to access the relationship between apoptosis rate and serum zinc, iron and copper levels. Mean number of apoptotic (TUNEL positive) cells, serum zinc, iron and copper levels were 2.15±0.22, 111.47±2.18 (μg/dl), 145.20±5.24 (μg/dl) and 137.52±3.04 (μg/dl) respectively. The present study found no relationship between the rate of apoptosis and baseline serum levels of neither of zinc, iron and copper. Our study showed that serum zinc, iron and copper levels didn't affect apoptosis in human gastric mucosa. It is suggested that more interventional and controlled studies be done.

Key words: Zinc, iron, copper, apoptosis, gastric mucosa

INTRODUCTION

Gastric cancer is so prevalent all around the world. Although, the prevalence of this cancer has decreased in the last 70 years, it is still the fourth most common malignancy in the world and the second leading cause of mortality among cancer patients. Incidence of Gastric cancer is particularly high in East Asia, Eastern Europe and parts of Central and South America (Brenner *et al*., 2009).

According to a population-based cancer registry, among the Middle East countries Iran has the highest rate of gastric cancer (Mohagheghi *et al*., 2009).

Sporadic gastric cancer is the result of genotypic changes due to an adverse environment (i.e. diet and Helicobacter pylori) (Nardone and Compare, 2008; Liu *et al*., 2009). Dietary habits and intake of nutrients play an important role in the both prevention and causation of gastric cancer (Pelucchi *et al*., 2009).

The biochemistry of iron, copper and zinc suggests that these metals may play an essential role in carcinogenesis (Toyokuni, 1996; Lauffer, 1992; Johnson and Fischer, 1992; Linder and Hazegh-Azam, 1996; Stevens and Kalkwarf, 1990; Swauger *et al*., 1991; Massa and Giulivi, 1993; Clogg *et al*., 1989; Kaim and Schwederski, 1994; Burke and Fenton, 1985; Prasad, 1983; Duchateau *et al*., 1981). Numerous studies (Stevens *et al*., 1994; Merk *et al*., 1990; Stevens *et al*., 1986; Selby and Friedman, 1988; Hann *et al*., 1989; Nelson *et al*., 1994; Herrinton *et al*., 1995; Akiba *et al*., 1991; Huang *et al*., 1999; Punsar *et al*., 1975; Schrauzer *et al*., 1977; Halsted and Smith, 1970; Margalioth *et al*., 1983; Magalora *et al*., 1999; Jayadeep *et al*., 1997; Haines *et al*., 1982) examined the relation of these metals with cancer risk in humans and many found a significant relationship between them. But the evidence linking iron, copper and zinc to cancer is far from conclusive.

Carcinogenesis is a complex process in which a number of mechanisms including programmed cell death or apoptosis might be involved (Kok *et al*., 1988; Bir *et al*., 2007). Moreover, it has been shown that apoptosis plays a fundamental role in precancerous changes in the gastric mucosa (Yang *et al*., 2006).

Apoptosis is an early indicator of carcinogenesis in gastric mucosa. This study was thus conducted to investigate the association between serum levels of zinc, iron and copper and apoptosis in gastric mucosa of patients undergoing upper gastrointestinal endoscopy.

Corresponding Author: Beitullah Alipour, Department of Nutrition, Faculty of Health and Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran

MATERIALS AND METHODS

This cross-sectional study was carried out in Tabriz Shahid Madani and Imam Reza hospitals; a city located in the north west of Iran between October and December 2008.

The patients over 18 years of age, referred to 2 main hospitals in Tabriz to undergo an upper gastrointestinal endoscopy were asked to participate in this study. A check list was completed for the patients willing to participate in the study, to exclude the ones not meeting the inclusion criteria. A total of 98 subjects out of 109 interested patients were eligible for the study. The exclusion criteria were as follows:

Alcohol consumption, smoking, any type of cancer, gastric surgery, certain cancer syndromes (hereditary nonpolyposis colorectal cancer, familial adenomatous polyposis, Peutz Jeghers syndrome), familial gastric cancer (defined according to the criteria proposed by International Gastric Cancer Linkage Consortium), gastric polyp (detected either in previous or present endoscopy), Menetrier's disease, Pernicious anemia and non-elective endoscopy.

Participants included were given a brief description on the goals and the procedure of the study and a written informed consent was acquired from all the subjects.

A skilled gastroenterologist who was completely familiar with the inclusion criteria of the study performed an upper GI endoscopy. On detection of any suspicious lesion or tumor during the endoscopy, the subject was excluded from study. Otherwise, two biopsy samples from the antrum were taken by the physician and one of which was immediately put in formalin 20% and sent to clinical pathology laboratory to be studied by a skilled pathologist. The second sample was kept in buffered formalin 10% and was taken to histology laboratory in faculty of medicine, Tabriz university of Medical Sciences, for detecting apoptosis by TUNEL (terminal deoxynucleotidyl transferase nick-end labeling) technique.

After the endoscopy was performed, the patient was lead to another room where a trained nutritionist completed a demographic questionnaire for him/her when relaxed and ready.

Anthropometric measurements were also performed and Body Mass Index (BMI) was calculated as: BMI = weight (kg)/stature $(m)^2$.

After 12 h of fasting, blood samples were collected and centrifuged at 4°C and 500 rpm for 10 min to separate the sera which were then transferred to a-80°C freezer until being analyzed for zinc, iron and copper. Levels of zinc, iron and copper were measured by atomic absorption spectroscopy. Quality control was strictly carried out using standard reference materials.

Infection with Helicobacter Pylori (HP) affects the rate of apoptosis in gastric mucosa. Approximately 2% of epithelial cells in the normal stomach are apoptotic. In gastritis by HP-infection, epithelial proliferation and apoptosis are moderately increased, with approximately 8% apoptotic epithelial cells (Xu *et al*., 2001; Herbay and Rudi, 2000).

On the other hand infection with HP is common in Iran and the rate of infection has been reported to be 69-89% in different parts of the country (Malekzadeh *et al*., 2009). This decided us to examine apoptosis in patients with HP-induced chronic nonspecific gastritis in the antrum. 62 patients had the very criterion of having HP-induced gastritis in which HP infection could be omitted as a confounding factor on apoptosis. These 62 subjects entered the final phase of the study.

Apoptosis was assessed by the terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate end labeling (TUNEL) method.

In this phase, sections (4 μm thick) were cut from paraffin embedded blocks and mounted on microscope slides. The slides were then incubated in 37°C for 36 h before being deparaffinized and rehydrated. Next the sections were deparaffinized, rehydrated in graded alcohol series and incubated with proteinase K (Roche diagnostics, Germany) in 37°C for 30 min. TUNEL solution (Roche diagnostics, Germany) was prepared according to the instruction manual and the samples were incubated with this solution for 1 h in 37°C. After washing, POD solution (Roche diagnostics, Germany) was added and the slides were incubated for another 30 min in 37°C.

Then DAB (di-amino banzidil) solution (Roche diagnostics, Germany) was added and after 15 minutes in room temperature, the slides were washed and counter stained with methylene blue. The prepared slides were examined under light microscopy (Nikon x 40). The number of apoptotic cells was counted in 10 High Power Fields (HPF) for each slide and the mean number of apoptotic cells for each section was calculated. The study was approved by the ethics committee of Tabriz University of Medical Sciences.

For all continuous variables normality was tested by Q-Q test. All values are expressed as Means±SE at each time interval. We used t-test for compare of means (male, female) and Stepwise regression was exploited to access the relationship between apoptosis rate and serum zinc, iron and copper.

The level of significance was set at $p<0.05$. Data was analyzed by SPSS version 16.00.

RESULTS

62 subjects were finally enrolled in this study. Figure 1 shows the flow chart of the participants of the study.

Some of the general characteristics of the patients (mean±SE) including age, BMI, serum zinc, iron and

Table 1: The general characteristics of participants and serum level of zinc, iron and copper in male and female

	Mean±SE (Female)	Mean±SE (male)	Pv
Age (year)	42.87±2.15	45.09±2.54	0.50
BMI (kg/m^2)	25.60±0.59	25.00±0.71	0.52
Zinc (µg/dl)	112.51±3.11	111.00±3.14	0.73
Iron (µg/dl)	145.74±7.44	145.16±7.73	0.96
Copper (µgl/dl)	145.54±4.25	130.16±3.97	0.01
Apoptosis	2.25±0.33	2.01±0.29	0.58

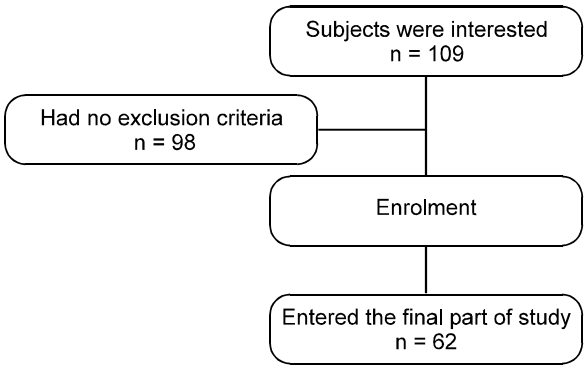

Fig. 1: Study flow diagram of participants

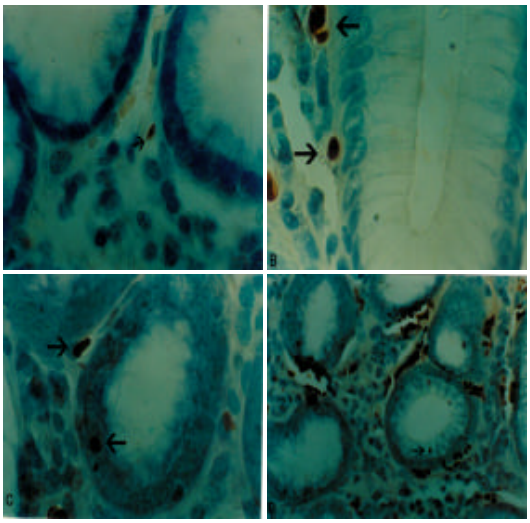

Fig. 2: Apoptosis in human gastric mucosa. The arrows show apoptotic cells

copper and apoptosis are shown in (Table 1) for men and women separately. Apoptotic cells are shown in Fig. 2.

Total Mean number of apoptotic (TUNEL positive) cells, serum zinc, iron and copper levels were 2.15±0.22, 111.47±2.18 (µg/dl), 145.20±5.24 (µg/dl), 137.52±3.04 (µg/dl) respectively.

Mean serum copper levels were significantly different for the two genders (Table 1) ($p<0.05$). No significant differences were observed for the other parameters.

Having employed Stepwise regression, no relationship was found between the serum levels of zinc, iron and copper and apoptosis of gastric cells.

DISCUSSION

This was the first study to examine the relationship between serum zinc, iron and copper levels and apoptosis in gastric mucosa. In this cross-sectional study we found no relationship between serum zinc, iron and copper and occurrence of apoptosis in gastric mucosa.

Iron, copper and zinc may play an important role in carcinogenesis (Toyokuni, 1996; Lauffer, 1992; Johnson and Fischer, 1992; Linder and Hazegh-Azam, 1996; Stevens and Kalkwarf, 1990; Swauger *et al*., 1991; Massa and Giulivi, 1993; Clogg *et al*., 1989; Kaim and Schwederski, 1994; Burke and Fenton, 1985; Prasad, 1983; Duchateau *et al*., 1981). Several possible mechanisms have been proposed for the probable role of iron, copper and zinc in cancer etiology. As transition metals, iron and copper can produce the reactive oxygen species such as hydroxyl radicals (Toyokuni, 1996; Lauffer, 1992; Johnson and Fischer, 1992; Linder and Hazegh-Azam, 1996). Reactive oxygen species can attack DNA and cause DNA mutation, which is an element in the pathological process of cancer. On the other hand, iron may be a limiting nutrient to the growth and replication of cancer cells in the human (Stevens and Kalkwarf, 1990). Copper has been concerned in the activation of several organic peroxides and making them more carcinogenic (Swauger *et al*., 1991; Massa and Giulivi, 1993). On the contrary, zinc may play an anti-carcinogenic role by stabilizing the structure of DNA, RNA and ribosome (Clogg *et al*., 1989). Zinc is also necessary to the functions of several transcription factors, proteins that recognize certain DNA sequences and control gene transcription (Kaim and Schwederski, 1994). Zinc protects against free radical damage (Burke and Fenton, 1985) and may influence immune response (Prasad, 1983; Duchateau *et al*., 1981).

No significant differences in the rate of apoptosis for subjects with low or high baseline levels of serum iron, observed in this study is inconsistent with the findings from many human studies in which an association between higher circulating iron levels and increased risk of cancer has been reported (Stevens *et al*., 1988; Stevens *et al*., 1994; Knekt *et al*., 1994; Merk *et al*., 1990; Stevens *et al*., 1986; Selby and Friedman, 1988; Hann *et al*., 1989; Nelson *et al*., 1994).

Weinberg (1996) has written a general description of the important role of iron in the development of cancer. Tumors grow better in an iron-rich condition. In an animal study conducted in 1989, 19 rats were injected daily iron for 3 months. Nine animals developed tumors while all animals in the control group with no iron injection, remained free of tumors (Okada *et al*., 1989).

There are a few studies (Herrinton *et al*., 1995; Akiba *et al*., 1991) which our findings are in agreement with. In a prospective study of 38,538 people who had an average of 17.7 years of age, no significant association was observed between transferrin saturation and epithelial, lung, or stomach cancer incidence in neither men nor women (Herrinton *et al*., 1995). However, taking serum ferritin and hemoglobin levels into account may be crucial to confirming these results and drawing an evidential conclusion.

In our study no significant differences were found in the rate of gastric mucosa apoptosis for subjects with low or high baseline levels of serum copper and zinc. This is inconsistent with the finding from most of the earlier studies which have reported higher serum copper levels in the cases of cancer (breast cancer, hepatic cancer and gastroesophageal cancer) in comparison to the controls (Haung *et al*., 1999).

The roles of copper and zinc in human cancer etiology are much less studied. Cross-sectional and case-control investigations (Punsar *et al*., 1975; Schrauzer *et al*., 1977; Halsted and Smith, 1970; Margalioth *et al*., 1983; Magalora *et al*., 1999; Jayadeep *et al*., 1997; Huang *et al*., 1999) have shown higher serum copper and/or lower serum zinc levels in cancer patients. But the results of our study were in contrast with many studies. Many *in vitro* studies (Sunderman, 1995; Fraker and Telford, 1997; Truong-Tran *et al*., 2000) have shown that apoptosis is induced in a variety of cell types when zinc is depleted by chelators and infertiled by high concentrations of zinc (500-1000 M). With lower or more physiologic concentrations of zinc, Fraker and Telford (1997) confirmed that zinc could actually induce death in various types of cells. Thus, zinc may be a modulator of apoptosis.

In the present study, we showed that there is no significant relationship between serum zinc, iron and copper and apoptosis in gastric mucosa. Confliction of our results to those of previous studies are probably due to the different effects of zinc, iron and copper on carcinogenesis different cell types. Having accepted a key role for these metals in cancer development, altering apoptosis rate is just one mechanism through which they may control cell proliferation. Therefore focusing on mechanisms other than apoptosis, by which zinc, iron and copper could affect carcinogenesis, may be of great aid in understanding the association between these metals and gastric cancer. In other words our findings which indicated no relationship between serum zinc, iron and copper levels and apoptosis in gastric mucosa, do not necessarily mean that these metals are not of any significance in controlling gastric cancer. Another possible explanation for our results might be that different types of HP might interact differently with the effects of zinc, iron and copper and therefore it might be mandatory to match the subjects according to their HP subtype before assessing the relationship between these metals and apoptosis in gastric mucosa.

The advantage of the present study was its statistical method. The concurrency of analysis in this study minimized the probability of false positive results and this may be one reason our results were not in agreement with many other studies.

Our study had several limitations. Because cell proliferation and cell death are two sides of a coin both of which influence the process of carcinogenesis, assessing cell proliferation in addition to apoptosis rate could have strengthened our study. Furthermore in spite of the fact that TUNEL is a very well known method for detecting apoptosis, it suffers from some shortcomings. So apoptosis detection exploiting TUNEL has false positives and false negatives. Another limitation was that many factors including other vitamins and minerals can effects apoptosis. Moreover because of the limitations of measuring serum zinc, iron and copper, concentration of these metals in epithelial cells have been advocated as a more reliable method for the measurement of these metals. Larger interventional and controlled studies taking into account the effects of other micronutrients are suggested to characterize the relation between these metals and gastric cancer risk.

Conclusion: Our study showed that there is no significant relation between levels of zinc, iron and copper in serum and apoptosis in human gastric mucosa. As apoptosis is one of the effective components in the process of gastric cancer, it is worth designing more elaborate prospective and interventional studies which will make it possible to examine these findings more specifically. Studies performed under more controlled conditions such as cell culture studies, measuring level of these metals in epithelial cells, measuring the rate of gastric cell proliferation and finding better methods for detecting apoptosis will be very helpful in determining the effect of certain nutrients on apoptosis.

ACKNOWLEDGEMENTS

This study was supported by Tabriz University of Medical Sciences, Nutrition Research Center and Liver and Gastrointestinal Disease Research Center. We thank the participants of this study for their enthusiastic support.

REFERENCES

Akiba, S., K. Neriishi, W.T. Blot, M. Kabuto, R.G. Stevens, H. Kato and E.C. Land, 1991. Serum ferritin and stomach cancer risk among a Japanese population. Cancer, 67: 1708-1712.

Bir, F., N. Calli-Demirkan, A.C. Tufan, M. Akbulut and N.L. Satiroglu-Tufan, 2007. Apoptotic cell death and its relationship to gastric carcinogenesis. World J. Gastroenterol, 13: 3183-3188.

Brenner, H., D. Rothenbacher and V. Arndt, 2009. Epidemiology of stomach cancer. Methods Mol. Bio., 472: 467-477.

Burke, J.P. and M.R. Fenton, 1985. Effect of a zinc-deficient diet on lipid peroxidation in liver and tumor subcellular membranes. P Soc. Exp. Biol. Med., 179: 187-197.

Clogg, M.S., C.L. Keen and I.S. Hurley, 1989. Biochemical pathologies of zinc deficiencies. In: Mills, CF. (Eds.), Zinc in Human Biology. International Life Science Institute, London.

Duchateau, J., G. Delespesse, R. Vrijens and P. Collet, 1981. Beneficial effects of oral zinc supplementation on the immune response of old people. Am. J. Med., 70: 1001-1004.

Fraker, P.J. and W.G. Telford, 1997. A reappraisal of the role of zinc in life and death decisions of cells. Proc. Soc. Exp. Biol. Med., 215: 229-236.

Haines, A.P., A.P. Thompson, T.K. Basu and R. Hunt, 1982. Cancer, retinal binding protein, zinc and copper. Lancet, 1: 52-53.

Halsted, J.A. and J.C. Smith, 1970. Plasma-zinc in health and disease. Lancet, 1: 322-324.

Hann, H.W.L., C.Y. Kim, W.T. London and B.S. Blumberg, 1989. Increased serum ferritin in chronic liver disease: A risk factor for primary hepatocellular carcinoma. Int. J. Cancer, 43: 376-379.

Herbay, A. and J. Rudi, 2000. Role of apoptosis in gastric epithelial turnover. Microscopy Res. Tech., 48: 303-311.

Herrinton, L.J., G.D. Friedman, D. Baer and J.V. Selby, 1995. Transferrin saturation and risk of cancer. Am. J. Epidemiol., 142: 692-698.

Huang, Y.L., J.Y. Sheu and T.H. Lin, 1999. Association between oxidative stress and changes of trace elements in patients with breast cancer. Clin. Biochem., 32: 131-136.

Jayadeep, A., P.K. Raveendran, S. Kannan, K.R. Nalinakumari, B. Mathew, N.M. Krishnan and V.P. Menum, 1997. Serum levels of copper, zinc, iron and ceruplasmin in oral leukoplakia and squamous cell carcinoma. J. Exp. Clin. Cancer Res., 16: 295-300.

Johnson, M.A. and J.G. Fischer, 1992. Is copper an antioxidant nutrient? Crit. Rev. Food Sci. Nutr., 32: 1-31.

Kaim, W. and B. Schwederski, 1994. Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life. John Wiley and Sons, New York.

Knekt, P., H. Reunanen, H. Takkunen, A. Aroma, M. Heliovara and T. Hakulinen, 1994. Body iron stores and risk of cancer. Int. J. Cancer, 56: 379-382.

Kok, F.J., C.M.V. Duijn, A. Hofman, G.B. Van Det Voet, F.A. Wolff, C.H. Paays and H.A. Valkenburg, 1988. Serum copper and zinc and the risk of death from cancer and cardiovascular disease. Am. J. Epidemiol., 128: 352-359.

Lauffer, R.B., 1992. Iron and Human Disease. Ann Arbor, CRC Press.

Linder, M.C. and M. Hazegh-Azam, 1996. Copper biochemistry and molecular biology. Am. J. Clin. Nutr., 63: 797s-811s.

Liu, H., D.S. Merrell, C. Semino-Mora, M. Goldman, A. Rahman, S. Mogs and A. Dubois, 2009. Diet synergistically affects helicobacter pylori-induced gastric carcinogenesis in nonhuman primates. Gastroenterology, 137: 1367-1379.

Magalora, T., V. Bella, A. Brtkova, I. Beno, M. Kudlackova and K. Volkovova, 1999. Copper, zinc, super oxide dismutase in precancerous, benign disease and gastric, colorectal and breast cancer. Neoplasma, 46: 100-104.

Malekzadeh, R., M.H. Derakhshan and Z. Malekzadeh, 2009. Gastric cancer in Iran: Epidemiology and risk factors. Arch. Iran Med., 12: 576-583.

Margalioth, E.J., J.G. Schenker and M. Chevion, 1983. Copper and zinc levels in normal and malignant tissues. Cancer, 52: 868-872.

Massa, E.M. and C. Giulivi, 1993. Alkoxyl and methyl radical formation during cleavage of tert-butyl hydroperoxide by a mitochondrial membrane-band redox active copper pool: An EPP study. Free Radic. Biol. Med., 14: 559-565.

Merk, K., B. Mattsson, A. Mattsson, G. Hold, B. Gullbring and M. Bjorkholm, 1990. The incidence of cancer among blood donors. Int. J. Epidemiol., 19: 505-509.

Mohagheghi, M.A., A. Mosavi-Jarrahi, R. Malekzadeh and M. Parkin, 2009. Cancer incidence in Tehran Metropolis: The forth report from the Tehran population-based cancer registry, 1998-2001. Arch. Iran. Med., 12: 15-23.

Nardone, G. and D. Compare, 2008. Epigenetic alterations due to diet and Helicobacter pylori infection in gastric carcinogenesis. Expert Rev. Gastroenterol. Hepatol., 2: 243-248.

Nelson, K.G., F.G. Davis, E. Sutter, L.H. Sobin, J.W. Kikendall and P. Bowen, 1994. Body iron stores and risk of colonic neoplasia. J. Natl. Cancer Inst., 86: 455-460.

Okada, S., S. Hamazaki, S. Toyokuni and O. Midorikawa, 1989. Induction of mesothalioma by intraperitoneal injection of ferric saccharate in male Wislar rats. Br. J. Cancer, 60: 708-711.

Pelucchi, C., I. Tramacere, P. Bertuccio, A. Tavani, E. Negri and C.L. Vecchia, 2009. Dietary intake of selected micronutrients and gastric cancer risk: An Italian case-control study. Ann. Oncol., 20: 160-165.

Prasad, A.S., 1983. Clinical, biochemical and nutritional spectrum of zinc deficiency in human subjects: An update. Nutr. Rev., 41: 187-208.

Punsar, S., O. Erametse, M.J. Karuonen, A. Ryhanen, P. Hilska and H. Vomano, 1975. Coronary heart disease and drinking water. J. Chronic Dis., 28: 259-287.

Schrauzer, G.N., D.A. White and C.J. Schneider, 1977. Cancer mortality correlation studies. IV. Associations with dietary intakes and blood levels of certain trace elements, notably se-antagonists. Bioinorg. Chem., 7: 35-56.

Selby, J.V. and G.D. Friedman, 1988. Epidemiologic evidence of an association between body iron stores and risk of cancer. Int. J. Cancer, 41: 677-682.

Stevens, R.G., R.P. Beasley and B.S. Blumberg, 1986. Iron-binding proteins and risk of cancer in Taiwan. J. Natl. Cancer Inst., 76: 605-610.

Stevens, R.G., B.I. Graubard, M.S. Micozzi, K. Neriishi and B.S. Blumberg, 1994. Moderate elevation of body iron level and increase risk of cancer occurrence and death. Int. J. Cancer, 56: 364-369.

Stevens, R.G., D.Y. Jones, M.S. Micozzi and P.R. Taylor, 1988. Body iron and the risk of cancer. N Engl. J. Med., 319: 1047-1049.

Stevens, R.G. and D.R. Kalkwarf, 1990. Iron, radiation and cancer. Environ. Health Persp., 87: 291-300.

Sunderman, F.W. Jr., 1995. The influence of zinc on apoptosis. Ann. Clin. Lab. Sci., 25: 134-142.

Swauger, J.E., P.M. Dolan, J.L. Zweier, P. Kuppusamy and T.W. Kensler, 1991. Role of the benzoyloxyl radical in DNA damage mediated by benzoyl peroxide. Chem. Res. Toxicol., 4: 223-228.

Toyokuni, S., 1996. Iron-induced carcinogenesis: The role of Redox regulation. Free Radic. Biol. Med., 20: 553-566.

Truong-Tran, A.Q., L.H. Ho, F. Chai and P.D. Zalewski, 2000. Cellular zinc fluxes and the regulation of apoptosis/gene-directed cell death. J. Nutr., 130: 1459S-66S.

Weinberg, E.D., 1996. The role of iron in cancer. Eur. J. Cancer Preuerition, 5: 19-36.

Xu, A.G., S.G. Li, J.H. Liu and A.H. Gan, 2001. Function of apoptosis and expression of the proteins Bcl-2, P53 and C-myc in the development of gastric cancer. World J. Gastroentero., 7: 403-406.

Yang, L., D.Y. Wu and Y. Xin, 2006. Doen regulation of caspase-3 expression in precancerous lesions and its relation to gastric carcinogenesis. Zhonghua Zhong liu Za Zhi, 28: 357-360.