African Journal of Biotechnology Vol. 11(4), pp. 974-978, 12 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2182
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Assessment of drug induced genotoxicity in gastric cancer patients

Madhuri K.[1], Vani K.[1], Rabbani Syed[2], S. Jithender Kumar Naik[1] and Khalid Alharbi

[1]Toxicology Laboratory, College for Women, Osmania University, Hyderabad, India.
[2]College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia.

Accepted 25 November, 2011

The aim of this study was to evaluate the frequency and severity of chromosomal damage during chemotherapy treatment, to understand the role of chemotherapeutic agents inducing genetic damage and to understand the phenomenon of acquiring drug resistance. Blood samples were collected from gastric cancer patients receiving chemotherapy with the cytotoxic drugs epirubicin, cisplatin and 5-fluorouracil, and 100 controls from recognized cancer hospitals under the supervision of an oncologist. Cytogenetic studies were carried out in peripheral blood lymphocytes of study population by adopting standard cytogenetic protocols such as (a) chromosomal aberrations (CA) and (b) sister chromatid exchanges (SCE). Student t- test was adopted to analyze the statistical significance. An increased pattern in the frequency of chromosomal aberrations was observed in the patients that received chemotherapy 10.14 ± 0.77 followed by 7.13 ± 0.48 without chemotherapy as against 3.70 ± 0.32 in control subjects ($p<0.001$). In conclusion, our results clearly reveal as a whole, the obvious effects of anticarcinogenic agents on the level of chromosomal aberrations in gastric cancer patients receiving therapy with respect to the genetic material.

Key words: Gastric cancer, cytogenetics, chromosomal aberrations, chemotherapy.

INTRODUCTION

Gastric cancer, commonly referred to as stomach cancer, can develop in any part of the stomach and may spread throughout the stomach and to other organs; particularly the esophagus, lungs, lymph nodes and the liver (WHO, 2009). Stomach cancer causes about 800,000 deaths worldwide per year. It is the fourth most common type of cancer and the second leading cause of cancer-related death in the world. Nearly million new gastric cancer cases, 9% of all cancers were diagnosed in the year 2002 alone (Ferlay et al., 2004).

Different chemotherapeutic regimens are in practice to combat this dreadful disease. The adjuvant therapies with different combinations of antineoplastic drugs are effective against a proliferating cell and hence patients are having longer survival time. Some of the commonly used anticancer compounds such as cisplatin, cyclophosphamide, epirubicin, 5-flourouracil, doxorubicin and etoposide with different combinations are in used for the treatment of gastric cancer. However, a standard combination chemotherapy regimen for gastric cancer has not been well established. Therapy resistance is the main cause of therapeutic failure and death in patients suffering from gastric carcinoma. Clinical resistance against systemic chemotherapy is likely to be multi-factorial and heterogenous. So far, no significant resistance factor that predicts the clinical outcome of systemic treatment of gastric carcinoma has been identified (Lage, 2003).

It is well established that some chemotherapeutic agents and radiation therapy generate reactive oxygen species (ROS) in patients during cancer therapy. Among the various models of action for related carcinogenesis, the oxidative stress mediated through reactive oxygen species (ROS) can directly or indirectly damage DNA,

*Corresponding author. E-mail: rabbanisyd@gmail.com

Table 1. Chromosomal aberrations frequencies in gastric cancer patients.

Parameter	Control	Cancer patients	Cancer patients with chemotherapy
No. of cases	100	60	70
Normal metaphases	963	5572	6290
Percentage of metaphases	96.30	92.86	89.85
Aberrant cell	37	428	710
Mean (S.D) aberrant cells	3.70 (0.32)	7.13 (0.48)	10.14 (0.77)

Data are mean (deviation) or number (%). * 100 Metaphases were scored for each sample.

lipids and proteins (Su et al., 2001; Kitchin and Ahmed, 2003; Manna et al., 2008). Major source of DNA damages leading to mutation and cancer are the reactions of DNA with ROS. The cellular response to ROS is complex and extremely cell and context dependent. The modulation of intracellular ROS levels in cells is important in controlling the development, and possibly the maintenance of tumors. The genetic instability and cytogenic alternations are perhaps more likely to be associated generation of ROS. The excess production of ROS which indeed decreases the antioxidant capacity of plasma (Wu et al., 2001) is the prime factor of induced genotoxicity and there by related carcinogenicity (Kessel et al., 2002).

Understanding the role of chemotherapeutic agents in inducing genetic damage may help us to understand the phenomenon of acquiring drug resistance. Genetic instability characterized by an abnormal number of chromosomes is a common feature of many human cancers and this genetic instability can be enhanced by exposure to chemotherapeutic drugs (Rush et al., 1997; Colella et al., 1999; Difilippantonio et al., 2000). The present study was done to evaluate the frequency and severity of chromosomal damage with chemotherapy treatment and also to understand the role of chemotherapeutic agents inducing genetic damage and the phenomenon of acquiring drug resistance.

MATERIALS AND METHODS

Selection of subjects

Blood samples were collected from 60 human subjects diagnosed with gastric carcinoma and another 70 human subjects with gastric cancers receiving chemotherapy in a combination of ECF (epirubicin, cisplatin and 5-fluorouracil) from recognized cancer hospitals under the supervision of an oncologist. The gastric cancer patients were receiving an adjuvant therapy in combination of the drugs in different cycles (the dosage information is obtained from the cancer hospital and also from the medical oncologist). The mean age group of the patients was in the range of 46 to 50 years and belonged to the same socio-economic status. Blood samples from 100 healthy individuals were collected as controls for the purpose of comparison.

All the participants were informed about the objective of the study and a written consent was obtained from each subject .The blood samples were collected and further manipulated in accordance with the recommendations of the bio-medical ethical guidelines. This study was approved by the Institutional ethics committee. Cytogenetic studies were carried out in peripheral blood lymphocytes of study population by adopting standard cytogenetic protocols such as (a) chromosomal aberrations (CA) and (b) sister chromatid exchanges (SCE)

Statistical method

The student t-test (Goldstein, 1965) was adopted to analyze the statistical significance in the frequency of mean chromosomal aberrations (CA) and sister chromatid exchanges (SCE) per cell between the control and the cancer patients. The use of student t-test for sister chromatid exchange is based on the assumptions that the number of sister chromatid exchange per cell is very small and it is following the Poisson distribution.

RESULTS

In the present study, analysis of chromosomal aberrations in the lymphocytes of gastric cancer patients was studied using standard metaphase analysis method. The difference in the incidence of chromosomal aberrations between the control, cancer patients and patients receiving the chemotherapy were subjected to statistical analysis and the values were found to be statistically significant at the level $p<0.05$.

The mean age of controls was 31.58 (9.4), gastric cancer patients was 34.5 (10.8) and gastric cancer patients undergoing chemotherapy was found to be 36.33 (8.62). Total frequency aberrations was found to be significantly higher with respect to the age in cancer patients when compared to controls ($p=0.03$). Baseline frequency of chromosomal aberrations was significantly higher among cancer patients when compared with controls. An increased pattern in the frequency of chromosomal aberrations was observed in the patients receiving the chemotherapy 10.14 (0.77) followed by 7.13 (0.48) in cancer patients as against 3.70 (0.32) in control subjects (Table 1).

Total CA observed in patients receiving the chemotherapy vs. cancer patients without therapy and mean total CA in patients receiving the chemotherapy was significantly higher when compared with cancer without chemotherapy ($\chi^2 = 46.51$, $p<0.001$). Total CA observed in patients receiving the chemotherapy vs. controls and mean total CA in patients receiving the chemotherapy

Table 2. χ^2 Analysis of chromosomal aberrations in human peripheral lymphocytes of the study group.

Group	χ^2 Value	P value
Control vs. cancer patients	12.59	0.0003
Control vs. cancer patients + chemotherapy	61.174	<0.001
Cancer patients vs. cancer patients + chemotherapy	467.515	<0.001

Table 3. Sister chromatid exchange frequencies in the study population.

Parameter	Control group	Cancer patients	Cancer patients with chemotherapy
No. of cases	100	60	70
Normal metaphases	3000	1800	2100
Total no. of SCE's	10345	13365	19338
SCE cell (mean ± SD)	3.45 (0.06)	7.42 (0.09)	9.20 (0.12)

Data are mean (deviation) or number (%). * 30 Metaphases were scored for each sample.

Table 4. χ^2 Analysis of SCE in human peripheral lymphocytes in the study group.

Group	χ^2 values	P value
Control vs. cancer patients	12.59	0.0003
Control vs. cancer patients + chemotherapy	39.001	<0.001
Cancer patients vs. cancer patients + chemotherapy	5.07	0.02

was significantly higher when compared with the controls (χ^2 = 61.174, p<0.001), and total CA observed in gastric cancer patients without chemotherapy vs. controls and mean total CA in gastric cancer without chemotherapy was significantly higher when compared with dcontrol (χ^2 = 12.59, p<0.003) (Table 2).

The percentage of mean sister chromatid exchange rate per cell in gastric cancer patient was 7.42 (0.09) followed by 9.20 (1.12) in gastric cancer patients receiving chemotherapy as against the 3.45 (0.06) in the control group (Table 3).

We found significant increase in the mean frequency of SCE in gastric cancer patients with chemotherapy when compared with the control (χ^2 = 39.001 p<0.001), total mean SCE in cancer without chemotherapy was significantly higher (χ^2=12.59 p.0003) as compared to the control. Simultaneously, a significant increase in mean SCE in gastric cancer with chemotherapy (χ^2 = 5.07, p<0.02) was seen as compared to the control (Table 4).Table 5 depicts an increase in frequency of gaps, breaks, dicentric and exchanges in CAs and isochromatid aberrations of gastric cancer patients receiving chemotherapy when compared with gastric cancer patients without chemotherapy and the controls.

DISCUSSION

Chromosomal aberrations in circulating human peripheral blood lymphocytes are well-established biomarkers in detection of genotoxicity. Cytogenetic analyses of lymphocytes from populations exposed to such agents show increased chromosomal aberration frequencies (Lazutka et al., 1999; Topaktas et al., 2002). The spectrum of aberrations includes mainly chromatid breaks, accentric fragments and dicentrics. The frequencies of aberrant cells in the cancer patients with therapy and without therapy were significantly high in comparison with the control group.

Thus, it clearly reveals the obvious effects of anticarcinogenic agents on the level of chromosomal aberrations in the cancer patients receiving therapy with respect to the genetic material as a whole.The present results are in agreement with that of Scheid et al. (1999) and Martin et al. (1989) who reported that the anticarcinogenic drugs induces the chromosomal aberrations in cancer patients.

Previous study conducted by De Nunez et al. (1984) showed a statistically significant increase in structural chromosome aberrations in patients with sporadic unilateral retinoblastoma. A significant chromosome change was reported by Schroder et al. (1981) in human chronic lymphocytic leukemia. Williams et al. (2005) reported increased frequencies of chromosomal aberrations in gastric tissue of gastric cancer patients which supports the present investigation.

Beckman and Angqvist, (1987) reported a drastic increase in numerical chromosomal aberrations in

Table 5. Classification of various chromosomal aberrations frequencies in the study population.

Parameter	Numberof cases	Chromatid aberrations				Iso- chromatid aberrations				Total
		Gaps	Breaks	Acentric fragments	Exchanges	Gaps	Breaks	Acentric fragments	Dicentrics	
Control	100	24 0.24 (1.31)	18 (0.18±0.70)	11 0.11 (0.00)	0 (0.00)	36 0.36 (2.52)	0 (0.00)	4 0.04 (0.07)	4 0.04 (0.07)	37 3.70 (0.32)
Cancer patients	60	100 1.66 (0.2)	94 1.56±014	109 1.81±0.25	11 0.18 (0.08)	62 1.03 (0.16)	29 0.48 (0.11)	78 1.30 (0.03)	107 1.78 (0.2)	428 7.13 (0.48)
Cancer patients with chemotherapy	70	142 2.02 (0.23)	154 2.20 (0.13)	165 2.35 (0.03)	19 0.27 (0.04)	134 1.91 (0.11)	46 0.65 (0.11)	156 2.22 (0.29)	162 2.31 (0.13)	710 10.14 (0.77)

Data are mean (deviation) or number (%).

esophageal adenocarcinoma patients.

In this study, chromatid and iso-chromatid gaps are taken into consideration. A higher frequency of dicentrics was recorded in the study and the dicentrics are typical and generally used as dosimeter for ionizing radiations. The dicentrics are known to be lethal in the cell proliferation, which implies the cancer risk that may originate from chromosomal aberrations. The exact reason for the higher frequencies of dicentrics recorded in the present study is not clearly known.

Evidences suggest that chromosomal changes may be intrinsically linked to cancer development. Chromosomal instability is characteristics of dysplasia and many pre-malignancy conditions, and specific chromosomal aberrations appeared to be associated with many types of cancers (Younis, 1986).

These abnormalities can activate oncogenes or result in the loss of tumor suppressor genes (Flier et al., 1998).

Although, the increased frequencies of chromosomal anomalies reported in this study could be associated with an increased risk, more accurate cytogenetic and epidemiological studies are needed to confirm this result.

REFERENCES

Beckman L, Angqvist KA (1987).On the mechanism behind the association between ABO *blood group and gastric carcinomas*. Hum. Hered. 37(3): 140-143.

Colella CS, Marchini M, D'Incalci, R, Brown MB (1999). Mismatch repair deficiency is associated with resistance to DNA minor groove alkylating agents, Br. J. Cancer, 80(3-4): 338-343.

De Nuñez M, Penchaszadeh VB, Pimentel E (1984). Chromosome fragility in patients *with sporadic unilateral retinoblastoma.* Cancer Genet Cytogenet. 11(2): 139-41.

Difilippantonio MJ, Zhu J, Chen HT (2000). DNA repair protein Ku80 suppresses chromosomal aberrations and malignant transformation. Nature, 404 : 510-514.

Ferlay J, Bray F, Pisani P, Parkin D (2002). Globocan Cancer Incidence, Mortality and Prevalence Worldwide 2004. IARC Cancer Base No. 5 Version 2.0 (Lyon, IARC Press).

Flier JS, Underhill LH, Friend SH, Dryja TP, Weinberg RA (1988). Oncogenes and tumor suppressor genes. N. Engl. J. Med. 318: 618-622.

Goldstein A (1965). Biostatistics.: An Introductory Text, 2nd ed. New York: The Macmillan Co.

Kessel M, Liu SX , Xu A, Santella R, Hei TK(2002). Arsenic induces oxidative *DNA damage in mammalian cells*. Mol. Cell Biochem. 234: 235-301.

Kitchin KT, Ahmed S (2003). Oxidative stress as a possible mode of action for *arsenic carcinogenesis*. Toxicol. Lett. 137: 3-13.

Lazutka JR, Lekevieius R, Dedonyte V, Maciulevieiute-Gervers I, Mierauskiene J, Rudaitiene S, Slapsyte G (1999). Chromosomal aberrations and sister chromatid exchanges in Lithuanian populations*: Effects of occupational and environmental exposures*. Mutat. Res. 445: 225- 239.

Lage H (2003). Molecular analysis of therapy resistance *in gastric cancer*. Dig. Dis. 21(4): 326-38.

Martine G, Marc S, Véronique R, Hiro O (1989). Immunotoxins Containing Glucose Oxidase and Lactoperoxidase with Tumoricidal Properties: In Vitro Killing Effectiveness in a Mouse Plasmacytoma Cell Model. Ito Cancer Res. 49: 5497-5504.

Manna P, Sinha M, sil PC (2008). Protection of arsenic-induced testicular oxidative stress by *arjunolic acid.* Redox rep. 13(2): 67-77

Rush EB, Rush EB, Tan LK (1997). Microsatellite instability *in breast cancer*. Ann. Surg. Oncol.. 4: 310-315

Scheid MP, Schubert KM, Duronio V (1999). Regulation of Bad phosphorylation and association *with Bcl-XL by the MAPK/Erk kinase.* J. Biol. Chem. 274: 31108-31113.

Su X Liu, Ather M,Lippai I, Waldren C, Hei TK (2001). Induction of oxyradicals by arsenic: implication of mechanism of genotoxicity. Proc. Natl. Acad. Sci. 98: 1643-1648

Schroder J, Vuopio P, Autio K (1981). Chromosome changes *in human chronic lymphocytic leukemia*. Cancer Genet Cytogenet. 4(1): 11-21.

Topaktas M, Renciizodullari E, Yla HB, Kayraldiz A (2002). Chromosomal aberration and sister chromatid exchange in

workers of iron and steel factory of Yskenderun, Turkey, Teratog, Carcinog. Mutagen, 22: 411-423.
Younis JJ (1986). Chromosomal rearrangements, genes and fragile sites in cancer- clinical and biological implications. In: Important advances in Oncology (De Vita Jr. VT, Hellman S, Rosenberg SA eds). Philladelphia PA. Lippincott. 1: 93-128
Williams, Jenkins GJS, Doak SH, Fowler P, Parry EM, Brown TH, Griffiths AP, Williams JG, Parry JM (2005). Fluorescence in situ hybridisation analysis of *chromosomal* aberrations in gastric tissue: the potential involvement of Helicobacter pylori. Br. J. Cancer, 92: 1759-1766
Wu MM, Chiou HY, Wang TW , Hsueh YM, Wang IH, Chen CJ, Lee TC (2001). Association of blood arsenic levels with increase reactive oxidants and decrease antioxidant capacity in a human population of North Eastern Taiwan. Environ. Health Presp.109: 1011-1017.
World Health Organization. Cancer (Fact sheet N°297)". http://www.who.int/mediacentre/factsheets/fs297/en/. Retrieved 2009-05-11.