

Full Length Research Paper

Assessment of drug induced genotoxicity in gastric cancer patients

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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-fluorouracil, 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 multifactorial 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,

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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 cytogenetic 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 \pm 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 ($\chi^2 = 61.174$, $p < 0.001$), 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 ($\chi^2 = 12.59$, $p < 0.003$) (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 ($\chi^2 = 39.001$ $p < 0.001$), total mean SCE in cancer without chemotherapy was significantly higher ($\chi^2 = 12.59$ $p = 0.0003$) as compared to the control. Simultaneously, a significant increase in mean SCE in gastric cancer with chemotherapy ($\chi^2 = 5.07$, $p < 0.02$) 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 anti-carcinogenic 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 | Number of 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±0.14 | 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.

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