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New Phenomenon

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Oxidative damage and cell signaling transduction in patients of chronic myeloid leukemia

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Chronic myeloid leukemia (CML) was the first human malignancy shown to be consistently associated with a chromosomal abnormality, the Philadelphia chromosome. At the gene level, the Philadelphia chromosome is the result of breaks on chromosomes 9 and 22 with a reciprocal translocation of the distal genetic material. This translocation forms the new hybrid BCR-ABL oncogene, an abnormal 8.5-kb RNA that encodes a 210-kDa fusion protein, which, presumably through its increased tyrosine kinase activity, changes normal hematopoietic cells into CML cells [1].

Oxidative stress is a biochemical condition that occurs when intracellular antioxidants are unable to neutralize pro-oxidants such as reactive oxidant species (ROS). These ROS damage membranes, DNA, lipids, proteins, and carbohydrates, eventually causing cell injury and death. ROS contribute to several cellular functions, including the regulation of signal transduction, gene expression, and cell proliferation [2].

Nuclear factor kappa B (NF-κB) is a redox-sensitive transcription factor that is mostly cytoplasmic and maintained in the cytoplasm by interaction with its inhibitor IκB-α. NF-κB pathway plays an important role in the control of cell proliferation, differentiation, apoptosis, stress response, cell signaling transduction, and other physiological processes [3]. ROS can activate NF-κB signal transduction pathways, which in turn lead to the transcription of genes involved in cell growth regulatory pathways.

A number of somatic mutations have been identified in cancer, like transversion mutations of p53. These key genes have been linked to cancer progression, and the mutations found in them can be produced by ROS. Although the direct link between ROS modification of DNA and mutations of these genes remains to be established, they should be considered important candidates for the induced carcinogenesis because mutations in these genes could be responsible for tumor initiation as well as progression. ROS have also been shown to increase the production of the angiogenic factor such as vascular endothelial growth factor (VEGF) in tumor cells resulting in carcinogenesis.

In an attempt to explore the possibility that cellular redox status may up-regulate NF-κB signaling, the present study was designed to correlate the alterations in the pro-oxidant/antioxidant status with the up-regulation of the nuclear transcription factor NF-κB, pro-inflammatory cytokine VEGF, and tumor suppressor gene p53 in the development/progression of CML.

Case control study consisted of 40 clinically diagnosed CML patients from the Department of Medicine, University Hospital, Banaras Hindu University (Varanasi, India), with cytogenetic analysis to confirm the presence of Philadelphia (Ph) chromosome. Exclusion criteria for enrolment in the study group were patients with clinical or pathologic evidence of cancer at any other site or having received any type of neo-adjuvant therapy along with the presence of liver dysfunction, diabetes mellitus, heart failure, or renal failure. The mean age of the CML patients was 36.7 ± 11.9 years. The mean leukocyte count was 80,410 per mm^3. The mean spleen size of patients measured by ultrasonography was 21.1 ± 3.5 cm, and was found to be significantly larger than controls. The Philadelphia chromosome was present in all of 40 patients with CML, which confirmed the BCR/ABL translocation. Forty age and sex-matched healthy volunteers having socioeconomic status similar to that of patients served as controls. None of the patients and controls was under antioxidant supplementation. The venous blood was drawn from patients and healthy volunteers in sterile tubes for various biochemical and hematologic investigations. The study protocol was approved by the ethical committee of the Institute of Medical Sciences, Banaras Hindu University. Informed consent of each patient and healthy volunteers was obtained purely for research purpose.

The oxidative stress parameters 8-hydroxydeoxyguanosine (8-OHdG), protein carbonyl (PC), and total antioxidant status (TAS) were measured [4–6]. NF-κB p65 subunit DNA-binding activity, VEGF, and p53 levels were estimated to correlate the up-regulation of nuclear transcription factor/cytokine level with increased oxidative stress so as to determine the role of oxidative microenvironment in the progression of disease. All the data were expressed as the mean ± standard error of the mean (SEM). Data were analyzed statistically by independent Student's *t*-test for comparison of parametric variables. Either linear or nonlinear regression analysis was applied for association studies. All statistical analyses were two-tailed and a value of

$P < 0.05$ was considered statistically significant. Odds ratios (ORs) and 95% confidence intervals (CIs) for CML risk in relation to markers of oxidative damage were estimated by using a logistic regression analysis.

Increased levels of 8-OHdG, PC, VEGF, and p53 and depleted antioxidant levels were found in patients in comparison with in healthy controls (**Supplementary Table S1**). Advanced disease and higher stage correlate with increased oxidative damage and decreased TAS. Serum VEGF and p53 in the CML patients were also demonstrated to be significantly associated with clinico-pathologic characteristics. A significant increase in DNA-binding activity of NF-κB p65 subunit was observed in patients with CML compared with controls (**Fig. 1**). The DNA-binding activity of NF-κB p65 subunit was also increased in advanced phase of disease (**Fig. 2**).

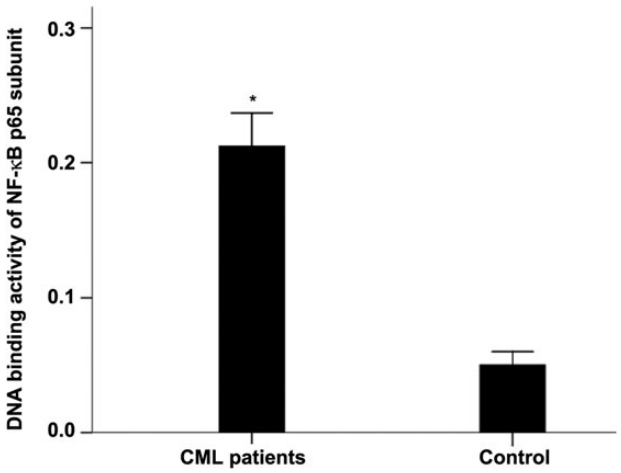

Figure 1. DNA-binding activity of NF-κB p65 subunit in CML patients and controls *$P < 0.05$ compared with controls.

Increased oxidative damage had a positive correlation with NF-κB, VEGF, and p53 (Table 1). Table 2 shows the ORs and 95% CIs for CML in relation to VEGF, p53, NF-κB, and oxidative damage markers. A positive association for risk of CML with 8-OHdG, PC, NF-κB, VEGF, and p53 was revealed, while a significant inverse correlation was found for CML carcinoma with TAS (Table 2).

Free radicals are implicated in the pathogenesis of tissue injury in many human diseases. The disturbance of the pro-oxidant/antioxidant balance, resulting from increased free-radical production, antioxidant enzyme inactivation, and excessive antioxidant consumption, is the causative factor in oxidative damage. A relationship between leukemia and oxidative stress has been reported. Leukemic cells produce higher amounts of ROS than non-leukemic cells because the former are experiencing sustained oxidative blockade [7]. In CML patients, the levels of 8-OHdG were found to be significantly higher than those in the normal healthy controls. The results reflect that oxidative damage is increased with advanced disease. Thus, the accumulation of pro-mutagenic oxidative DNA adducts in cells could ultimately result in genetic instability leading to the activation and dysregulation of well-recognized targets, such as oncogenes and tumor suppressor genes [8]. ROS exert their cytotoxic effect by carbonylating proteins, leading to a loss of protein function. Elevated levels of protein carbonylation products support the hypothesis that the cancer or malignant cells produce large numbers of ROS and that there exists a relationship between ROS activity and malignancy. Decreased levels of antioxidants occur as a consequence of oxidative insults as evidenced by the elevated levels of oxidative damage products in the circulation of CML patients [9]. The level of oxidative damage and reduced levels of antioxidants activity correlate positively with the increase in the patients with advanced disease, indicating an altered redox status in CML patients.

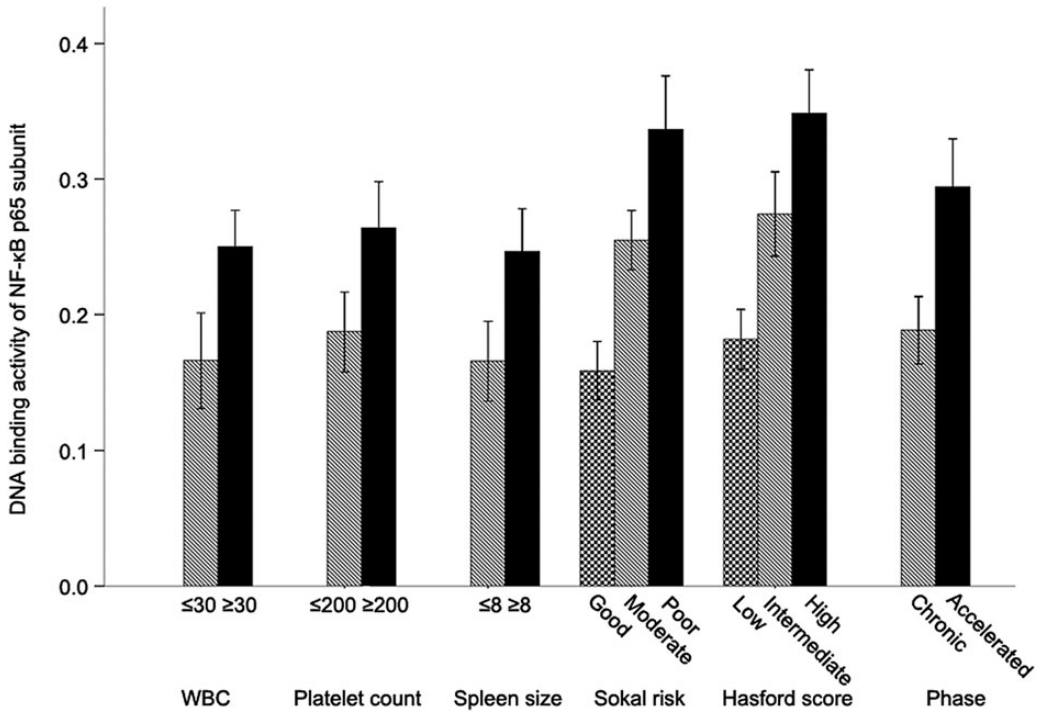

Figure 2. DNA-binding activity of NF-κB p65 subunit in relation to WBC count (10^9/l), platelet count (10^9/l), spleen size (cm), Sokal risk, Hasford score, and phase in CML patients

Table 1. Correlation between NF-κB, VEGF, and p53 with markers of oxidative stress in CML patients

Markers of oxidative stress	NF-κB	VEGF	p53
8-OHdG	$r = 0.97$ $P < 0.0001$	$r = 0.971$ $P < 0.0001$	$r = 0.939$ $P < 0.0001$
PC	$r = 0.926$ $P < 0.0001$	$r = 0.911$ $P < 0.0001$	$r = 0.829$ $P < 0.0001$
TAS	$r = -0.492$ $P < 0.001$	$r = -0.472$ $P < 0.005$	$r = -0.479$ $P < 0.005$

Table 2. Logistic regression analysis in CML patients

Serial number	Markers	β	OR	95% CI	*P*-Value
1	8-OHdG	0.021	1.022	1.010–1.033	<0.0001
2	PC	1.183	3.263	1.365–7.802	0.008
3	TAS	−1.348	0.260	0.025–0.315	0.015
4	VEGF	0.018	1.018	1.009–1.028	<0.0001
5	NF-κB	5.968	390.616	223.551–682.532	0.001
6	p53	0.045	1.046	1.041–1.051	0.002

Note: OR, odds ratio; 95% CI, 95% confidence interval; β, partial regression coefficient.

Research evidence has suggested that abnormalities in protein translation, modification (mainly phosphorylation), and degradation of DNA play critical roles in the initiation, development, and induction of the BCR/ABL transformation or increased levels of oxidative damage and decreased levels of the antioxidant system in the CML patients. Increased oxidative stress up-regulates NF-κB, which in turn regulates *VEGF* mRNA in CML cancer cells, supporting its role in angiogenesis and CML cancer metastasis. Under normal conditions, p53 levels are regulated by pathways that are activated in response to DNA damage or oncogene-induced cell proliferation. Mutations in p53 have been correlated with carcinogenesis and the growth of cancer. ROS can increase p53 activity either indirectly by producing DNA damage or directly by promoting p53 phosphorylation [10].

While exploring the association between the cellular redox status, NF-κB activation along with VEGF and p53 levels, a positive significant correlation among the prognostic indicators of CML was observed, which thereby suggest that the redox status does play a role in the progression of CML at advanced stages with the progression of disease. All the studied parameters bear association with the increased risk of disease.

Thus, we inferred that disturbance of the pro-oxidant/antioxidant balance would lead to an increased oxidative stress and that increased NF-κB activity, VEGF, and p53 levels altered by the cellular redox status might be associated with numerous factors in the development and progression of CML. Activation of NF-κB occurs in response to an increased ROS level, which promotes the dissociation of IκBs. Degradation of IκB proteins allows the entry of p65 subunit NF-κB into the nucleus where it binds to κB-regulatory elements. Decreased antioxidant level and increased NF-κB activation may promote angiogenesis through VEGF and p53. Further studies are needed to verify the exact mechanisms by which these factors function in the progression of disease.

Supplementary Data

Supplementary data is available at *ABBS* online.

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