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ORIGINAL ARTICLE

Assessment of Micronuclei Frequency in Sickled Tribal Population (Halba and Gond) of District Durg, Chhattisgarh, India

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ABSTRACT
Sickle cell disease is known to induce oxidative stress which is associated with increased production of oxidizing species leading to a variety of cytogenetic abnormalities. Micronuclei formation under the influence of oxidative stress under the influence of variety of toxicants is a well known fact. In the present study micronuclei frequency among sickled tribal (Halba & Gond) population (HbAS and HbSS) among different age groups (10-20, 20-30 and 30-40 years) has been estimated and compared with the control set of population from same age groups. The frequency of micronuclei was found significantly higher in both HbAS and HbSS population of all the corresponding age groups at P >0.05. Finding of the present work emphasize on genotoxicity among sickled population confirmed by micronuclei formation under influence of oxidative stress.

Keywords – Sickle Cell Anemia, Tribes, Oxidative Stress, Micronuclei

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INTRODUCTION

Sickle cell disorder is the most common heritable hemoglobin associated disease and in India, it is more prominent in the central and southern parts [1]. Several authors have emphasized about oxidative stress among sickle populations [2,3,4,5]. On another hand it has been also proved that genotoxicity and cytotoxicity are common phenomena under oxidative stress [6,7,8,9,10].

One parameter used in bio-indication is the generation of genetic material fragments in the form of Micronuclei, due to the activity of oxidative stress which provoke chromosome breaks. These fragments appear in the cytoplasm when either parts of the chromosomes or chromatids or entire chromosomes are not incorporated in the nuclei of the daughter cells in mitosis, frequently because these fragments do not have centromeres. These fragments left behind are incorporated in the secondary nuclei, called Micronuclei. Micronuclei have between 1/5 and 1/20 of the original nucleus' size and there is generally more than one per cell. Micronuclei test in red blood cells and lymphocytes can be used as an indicator of toxic effects in determined target populations, since DNA repair system is very sensitive to oxidative stress.

These micronuclei are the extra-nuclear DNA-containing entities that can be evaluated microscopically [11]. These entities are formed as a result of clastogenicity (chromosome breakage) and/ or chromosome loss [12]. Micronuclei reflect persisting chromosomal aberrations that may arise due to defects in chromosomal segregation or may be due to miss repair of DNA breaks. Increased levels of micronuclei in lymphocytes are generally associated with risk of cancers [13]. Micronuclei formation in the peripheral blood lymphocytes is a very well established method to study the chromosome damage in human population [14].

The method of micronuclei assay was first proposed by Countryman and Heddle [15] and was subsequently modified with the development of cytokinesis-block micronuclei method [16] and is now extensively used to analyze chromosome damage in human population. Micronuclei have been studied in mammalian cells for more than 20 years [17]. A substantial number of reports are available regarding

their formation in human lymphocytes and in different cell lines [18]. The micronucleus assay was first designed for exfoliated human cells [19]. Using this technique, micronucleus formation in exfoliated cells from the nose, mouth, bronchus and urothelial tract have been already investigated [20,21]. Many researchers have proved that micronuclei assay is an efficient and reliable tool for the investigation of clastogenic damage to the genetic material under the influence of oxidative stress imposing agents.
In the present study the micronuclei (MN) frequency was estimated in the lymphocytes of the peripheral blood lymphocyte cells of sickled tribal population (Halba & Gond) of Chhattisgarh, India, of different age groups (10-20, 20-30 and 30-40 years) to establish oxidative stress due to sickling can induce chromosomal aberrations leading to micronuclei formation and DNA damage.

MATERIAL AND METHODS

Sickled tribal population (Halba & Gond) of different age groups, selected from Durg District of Chhattisgarh, India, were categorized as HbAS, HbSS based of slide test and electrophoresis method from different age groups viz., 10-20 (n= 23, 13 HbAS & 10 HbSS), 20-30 (n= 21, 11 HbAS & 10 HbSS) and 30-40 years (n= 14; 10 HbAS & 4 HbSS) respectively. A control set in the form of 30 non-sickled subjects healthy individuals free from any ailments, chronic medical history and addictions (10 from each age group) were selected from the same community. Blood samples were collected from the subjects under study following a prior approval obtained from the Institutional Ethics committee (Approval No. – IEC/GVYTPGAC/02/DURG, Dt. 28.9.12). A prior written consent was also obtained from all participants enrolled for the study. The frequency of micronuclei was estimated following the method of Xue *et al.,* [22] (40µl of 0.3% methylcellulose solution added to 100µl of thawed blood followed by incubation at 37ºC for 40 minutes. Lymphocyte suspension thus prepared was then centrifuged at 100X g for 6 minutes. Pellets were mixed properly to prepare a cell suspension of lymphocytes. A small drop of the cell suspension was applied clean grease free glass slides to form a smeared film of the cell suspension. Sample was air dried and fixed with 100% methanol followed by staining with 7% buffered Giemsa stain and observed under 40x and then under 100x oil immersion objective lens). One thousand small lymphocytes per sample (both control and sickle positive) were scored.

RESULT AND DISCUSSION

The hypothesis of genotoxicity due to oxidative stress among sickled (HbAS & HbSS) population was verified by micronuclei test in lymphocyte cells (Figure 1 and 2). Among 10-20 years age group of tribal (Halba & Gond) population the average micronuclei per thousand lymphocytes was found significantly increased. In comparison to control among HbAS (2.4±0.70 in HbAA, 6.84±0.53 in HbAS, $t > 5.35$ at 5%P) it was found significant and in comparison to control among HbSS (2.4±0.70 in HbAA, 11.9±0.90 in HbSS, $t > 8.70$ at 5%P) it was reported highly significant (Table1; Figure3).

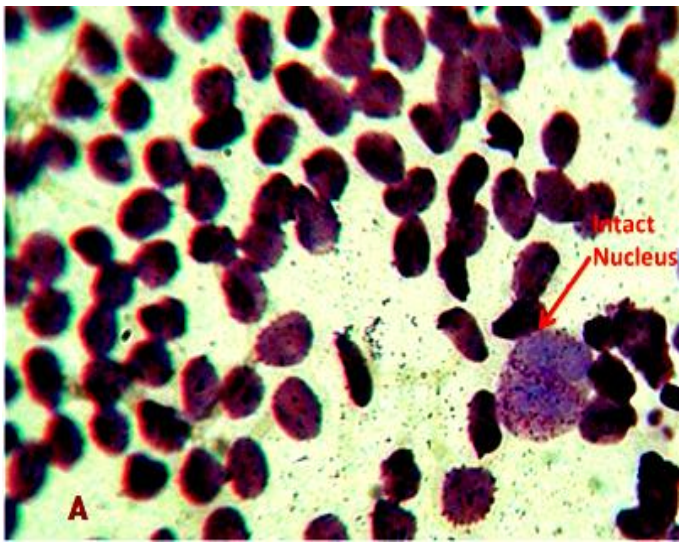

Figure1: Showing intact nucleus in lymphocyte cells of Control group (100x).

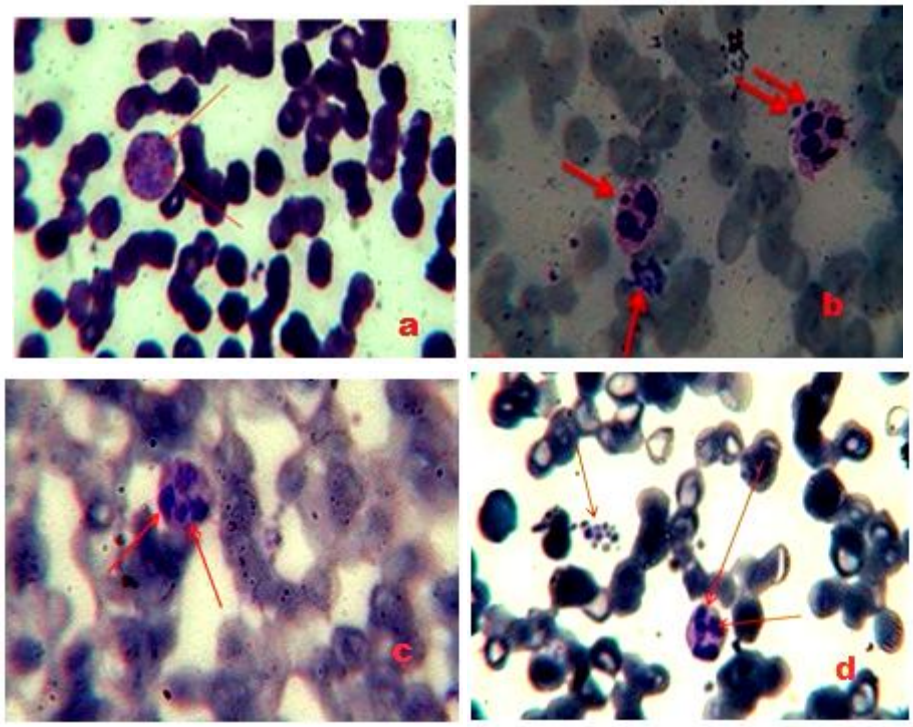

Figure2: (a,b,c,d) Micronucleated Lymphocyte cells of sickle cell anemia (HbAS & HbSS) affected individuals. (Red arrows indicates micronuclei).

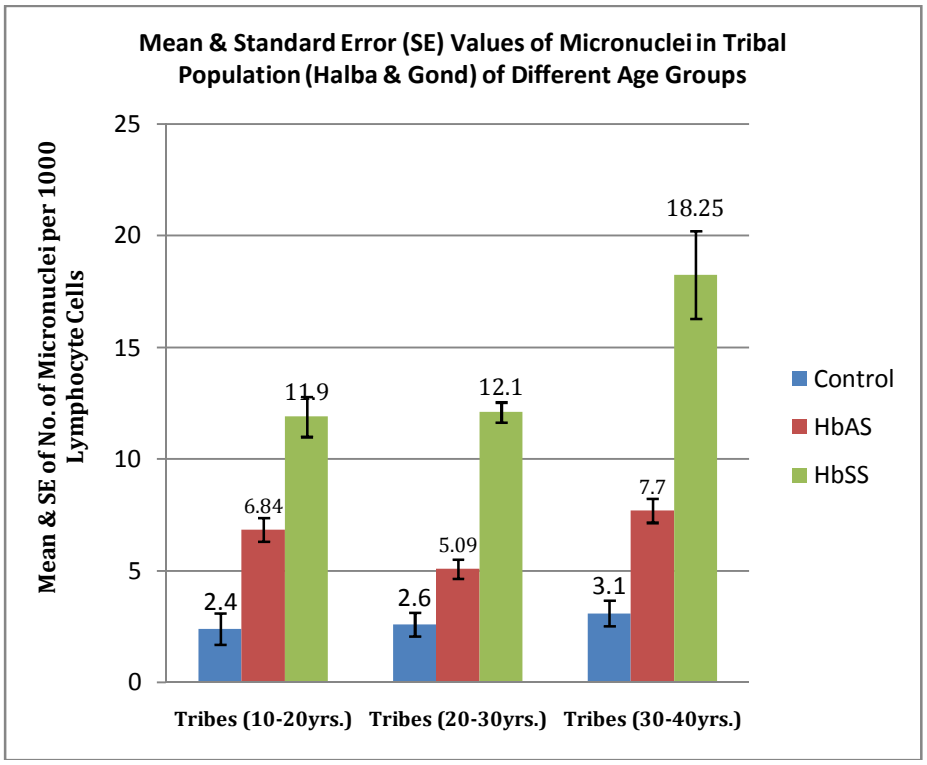

Figure 3: Showing micronuclei formation per 1000 lymphocyte cells among sickled tribal (Halba & Gond) population.

Table 1: Showing number of micronucleated cells per 1000 lymphocyte cells among sickled tribes (Halba & Gond) of different age groups

SNo.	Tribes Age Group 10-20yrs.			Tribes Age Group 20-30 yrs.			Tribes Age Group 30-40yrs.		
	Control HbAA (10)	HbAS (13)	HbSS (10)	Control HbAA (10)	HbAS (11)	HbSS (10)	Control HbAA (10)	HbAS (10)	HbSS (4)
1	3	8	10	2	7	14	0	5	17
2	4	6	12	3	6	12	4	8	23
3	0	9	16	0	4	11	3	8	15
4	4	9	15	4	6	11	4	9	18
5	0	4	12	3	3	12	5	8	-
6	0	7	7	0	6	13	0	9	-
7	4	6	9	5	3	11	4	8	-
8	5	6	12	4	5	12	3	5	-
9	4	5	12	2	6	12	4	7	-
10	0	9	14	3	4	13	4	10	-
11	-	7	-	-	6	-	-	-	-
12	-	4	-	-	-	-	-	-	-
13	-	9	-	-	-	-	-	-	-
Mean	**2.4**	**6.84**	**11.9**	**2.6**	**5.09**	**12.1**	**3.1**	**7.7**	**18.25**
SD	**2.11**	**1.86**	**2.72**	**1.64**	**1.37**	**0.99**	**1.72**	**1.63**	**3.40**
SE	**0.70**	**0.53**	**0.90**	**0.54**	**0.43**	**0.45**	**0.57**	**0.54**	**1.96**
t		**5.35**	**8.70**		**3.78**	**15.62**		**6.11**	**11.30**

Among 20-30 years age group, in comparison to Control among HbAS (2.6±0.54 in HbAA, 5.09±0.43 in HbAS, t > 3.78 at 5%P) it was found significant and in comparison to Control among HbSS (2.6±0.54 in HbAA, 12.1±0.45 in HbSS, t > 15.62 at 5% P) it was reported highly significant (Table1; Figure3).

Similarly among 30-40 years age group, in comparison to Control among HbAS (3.1±0.57 in HbAA, 7.7±0.54 in HbAS, t > 6.11 at 5% P) it was found significant and in comparison to Control among HbSS (3.1±0.57 in HbAA, 18.25±1.96 in HbSS, t > 11.36 at 5% P) it was reported highly significant (Table1; Figure3). Above findings confirm maximum impact of oxidative stress among HbSS than HbAA in term of micronuclei formation.

Everson *et al.,* [23], suggested that frequencies of micronuclei in the peripheral blood RBCs can be used to measure *in vivo* cytogenetic damage and their study provided a sensitive index of clastogenic damage and offered unique opportunities for investigation of the determinants of cytogenetic damage in humans.

In a study to determine that whether folate deficiency was linked with anemia, birth defects, cancer and neuropsychiatric disorders and a controlled changes in folate intake would affect chromosomal damage in lymphocytes and buccal cells or not [24], cytogenetic damage was assayed by scoring micronucleus (MN) frequency in lymphocytes and buccal cells and reported that the MN frequency was found increased in binucleated lymphocytes, as well as in all lymphocytes, after depletion (p=0.037), and decreased later following repletion. According to the authors the main variables affecting MN were vitamin B-12 level, plasma folate level, and baseline frequency of MN. The study indicated that low folate, without clinical symptoms of anemia, resulted in higher levels of cytogenetic damage in both the blood and oral cavity of postmenopausal women and micronuclei assay was significant method for such kind of studies.

Cytogenetic analysis performed in peripheral blood lymphocytes of hospital workers [25] that are chronically exposed to ionizing radiations (accumulated absorbed doses - 9.5 to 209.4 mSv) in comparison to matched non-exposed individuals in terms of chromosomal aberrations (CA), micronuclei (MN), and sister chromatid exchanges (SCE), reported significantly higher frequencies of chromosomal aberrations in the exposed individuals compared to the control group. In micronuclei analysis, they observed no significant (P=0.06) difference between both groups, but authors emphasized that the data should be cautiously interpreted since an increase in the frequencies of MN was found for radiation workers (3.0 MN/100 cells), compared to the control group (2.6 MN/100 cells) and that the increase occurred in parallel to CA and SCE frequencies.

Migliore *et al.,* [26] explored the relation between chromosome instability and oxidative stress biomarkers in Parkinson's disease (PD) using glutathione S-transferase activity in the plasma of affected patients along with the healthy controls and found that PD patients showed higher frequencies of micronuclei (17.2 +/- 4.8 vs. 9.0 +/- 3.4, $p < 0.001$) compared to the control individuals and a significant increased levels of single strand breaks (SSB) was also detected. Significant differences were also observed with respect to the distribution of oxidized purine bases between the two groups. Data obtained by fluorescence in situ hybridization analysis revealed that the percentage of centromere negative micronuclei is higher than that of centromere positive micronuclei. Glutathione S-transferase activity in plasma from PD patients and controls and the enzymatic activity in PD patients were found lower than in healthy controls. They concluded that oxidative stress could play a significant role as a risk factor in the etiology and pathogenesis of neurodegenerative diseases, and emphasized on the need for new individual and human-based approaches for further investigation of the problem.

Frequency of micronuclei formation in exfoliated cells of the buccal mucosa of 50 hairdressers (influenced by oxidative chromosomal damage due chemicals and substances used by them) and 50 controls was analyzed [27]. The authors carried out an assessment on the occurrence of micronulclei (MNC), binucleated cells (BNC), broken egg cells (BEC), budding cells (BC) and sum of anomalies (SA) in 2000 cells/ individual and statistically validated their results. According to their report the mean numbers of anomalies in all the cell types in the hairdressers were found significantly more as compared to the control subjects.

Santos *et al.,* [28] evaluated the *in vivo* genotoxicity of six (C1-C6) new lead compounds in test animals Swiss albino mice (25-30g) for the treatment of sickle cell disease through micronuclei test against commonly used drug hydroxyurea for the sickle cell disease. They reported that all the six compounds (C1-C6) induced an average frequency of less than six per 1000 cells at 12.5,25,50, and 100mg/kg of micronucleated reticulocyte cells in contrast to the hydroxyurea which induced an average micronucleated reticulocyte frequency of 7.8, 9.8, 15, and 33.7 per 1000 cells respectively at the same concentrations. They concluded that the compounds C1-C6 can be regarded as new non-genotoxic *in vivo* drug candidates for the treatment of sickle cell disease.

Our finding is affirmative to findings of previous authors and gives conclusion that oxidative stress among sickled population causes genotoxicity in terms of micronuclei formation, thus corresponding pathogenicity among sufferers.

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