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Baseline Frequencies of Spontaneous Chromosome Aberrations, Sister Chromatid Exchanges and Micronuclei in Human Newborns

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A cytogenetic monitoring programme on human newborns was carried out from 1983–1987 with a view to find out the incidence of constitutional chromosome anomalies. During this period studies were also undertaken to obtain baseline data on spontaneous chromosomal aberrations (CA), sister chromatid exchanges (SCEs) and micronuclei (MN) in limited number of human neonates. We report here the data on mean frequencies of spontaneous chromosome aberrations in the lymphocytes of 120 new borns, sister chromatid exchanges in 10 new borns and micronuclei in the lymphocytes of 6 new borns studied. The results are compared with data obtained in our laboratory on spontaneous chromosome aberrations and sister chromatid exchanges from adult blood lymphocytes. The micronuclei data is compared with the data from adult lymphocytes, by the cytokinesis blocked lymphocyte assay by cytochalasin B.

Materials and methods

Heparinised umbilical cord blood samples collected from BARC Hospital, Bombay were used in these investigations. Spontaneous chromosome aberrations were determined in 120 cord blood samples (collected from 72 male and 48 female newborn infants). Five cord blood samples each from male and female newborns were collected for determining the frequencies of SCEs and 3 cord blood samples each from male and female newborn infants were collected for determining the micronuclei frequencies in the lymphocytes by the conventional assay.

Lymphocyte cultures were set up within 24 hrs of collection by a standardised procedure of our laboratory. 4 ml Hams F 10 medium, 200 mM L. glutamine (Flow laboratories, U. K.) 0.5 ml foetal bovine serum (GIBCO, USA) 0.15 ml reconstituted PHA (Wellcome diagnostics, U. K.) and 0.3 ml whole blood. Cultures did not contain any antibiotics. Colcemid was added 2 hr prior to harvesting at a concentration of 0.02 μg/ml. Air dried preparations of hypotonically treated lymphocytes were made using routine techniques for chromosome analysis. A total of 100 metaphases per sample, no more than 50 metaphases per slide prepared from 48 hr cultures were analysed in the case of CA. All aberration types were included in the scoring. These were chromatid gaps, isochromatid gaps, chromatid breaks, isochromatid breaks and chromatid exchanges, among the chromatid types. Isochromatid gaps and breaks were considered as 2 gaps and breaks respectively. Among the chromosome types, dicentrics, centric rings and asymmetrical exchanges were scored.

The frequency of SCEs was analysed in second division metaphases from 72 hr cultures. The culture medium contained 20% heat inactivated human AB serum instead of foetal bovine serum. 5-bromo deoxyuridine (Brdu. Sigma, USA) was added at a final concentration of 10 μg/ml at the beginning of the cultures. For differential staining the hot salt treatment technique essentially according to Sakanishi and Takayama (1977) was followed. The slides were incubated in Na_2HPO_4 (1.0 M) at 75°C for 2 min (pH 8.8) rinsed briefly in distilled water

stained with Merck's Giemsa stain 4% for about 5–7 min. A total of 50 second cycle metaphases per sample, 25 metaphases per slide were analysed.

Micronuclei were scored in lymphocytes without colcemid pretreatment after mild hypotonic treatment and fixation from 48, 72 and 96 hr cultures. 2000 cells were analysed per subject in each series, 1000 cells per slide.

Since it may not always be possible to process cord blood samples immediately after collection, samples have to be stored for varying periods and transported, it is important to know the effect of storage duration on cultures. Cultures with good mitotic index (10–14%) could be routinely obtained from 5–6 day old cord blood samples stored at 4°C and even 7 day old blood yielded a mitotic index of 10%. Only constitutional chromosome anomalies were analysed from such cultures. Spontaneous CA, SCE and MN were analysed only in cord bloods cultured within 24 hrs of collection.

Results

The results obtained are presented in Table 1. The mean frequency of chromatid breaks per metaphase and chromatid gaps per metaphase, together with their standard errors and the range of values seen in the newborns in the study are given in Table 1. 120 cord blood samples analysed gave a mean frequency of 0.0052 breaks per cell (range 0–4) and 0.0059 gaps per cell (range 0–6) respectively. No statistically significant difference was noticed between male and female newborns in the spontaneous CA frequency. Only one dicentric chromosome was noticed. Neither ring chromosomes nor asymmetrical exchanges were seen in the cells analysed. Analysis of differentially stained chromosomes by the BrdUrd method in 48 hr cultures showed 14% second division metaphases. 20 adult blood samples 4 females and 16 males (age-wise breakup is given in Table 1) analysed in our laboratory gave mean frequency of 0.0085 chromatid breaks per metaphase and 0.0141 chromatid gaps per metaphase in 48 hr cultures. The values obtained in 72 hr cultures in 10 adult blood samples ranging in age from 44–49 are also given in Table 1.

The mean frequency of SCEs observed in 10 new borns was 5.56 ± 0.21 SCE/cell with a range of individual values from 4.40–6.60 SCE/cell. Values observed in single cells ranged from 2 to 11. No statistically significant influence was found of sex on the frequency of SCEs. A large statistically significant elevation was found when adult blood samples were compared to cord blood samples (difference 1.94 SCE/cell, $P<0.001$ by simple 't' test). In the adult blood samples (1 F and 4 M) the mean SCE value was 7.496 SCE per cell. Cell cycle analysis in cord bloods revealed 17–22% first division metaphases, 60–80% second division metaphases, 3–20% third division metaphases and 0–2% fourth division metaphases compared to adult blood samples, which revealed 17–38% first division metaphases 56–68% second division metaphases, 2–15% third division metaphases and 0–1% fourth division metaphases.

The frequency of micronuclei in cord blood lymphocytes was found to be 0–1.5 per 1000 cells in 48 hr cultures, 1–3 per 1000 cells in 72 hr cultures and 2–5 per 1000 cells in 96 hr cultures. The mean micronuclei yield by the cytokinesis block method in 10 adult blood samples was 0.0123 ± 0.001/cell.

Discussion

Hatcher and Hook (1981) reported the frequency of cells with chromosome aberrations to be 0.82% in cord bloods from normal babies. Various authors reported an aberration frequency of 0.6–3.2% in normal infants between 0–15 days old (Goodman *et al.* 1969, Bochkov 1972, Bregman *et al.* 1976). In the earlier studies aberration analysis was carried

Table 1. Frequencies of spontaneous chromosome aberrations, sister chromatid exchanges and micronuclei in cord blood lymphocytes of new borns and peripheral blood lymphocytes of adults

	No. of subjects	Culture duration	No. of metaphases/cells scored	Mean chromatid break/cell±S. E.	Range	Mean chromatid gap/cell±S. E.	Range
Spontaneous Chromosome aberrations	New borns 120	48h	12,000	0.0052±0.001	0–4	0.0059±0.001	0–6
	72 males		7,200	0.0053±0.001	0–4	0.0055±0.001	0–5
	48 females		4,800	0.0050±0.001	0–4	0.0064±0.001	0–6
	Adults 20 (20–60 Yrs) 4 females 16 males	48h	4,000	0.0085±0.001	0–4	0.0141±0.003	0–4
	Adults 10 (44–49 Yrs) 1 female 9 males	72h	1,200	0.013±0.004	0–4	0.019±0.004	0–4
				Mean SCE/cell±S. E.			
Sister chromatid exchanges	New borns 10	72h	500	5.56±0.21	2–11	P<0.001 compared with	
	5 males		250	5.59±0.36	2–11	Adults: non-smokers	
	5 females		250	5.53±0.27	2–11	('t' test)	
	Adults 5 1 female 4 males		250	7.50±0.21	2–17		
				Micronuclei/1000 cells Range		Micronuclei/CB cell±S. E.	
Micronuclei	New borns 6	48h	2000 cells	0–1.5			
	3 females	72h	per subject	1–3			
	3 males	96h		2–5			
	Adults 10 (25–50 Yrs) 8 males 2 females	72h	1000 cells per subject	Cytokinesis Block method		0.0123±0.001	

out in only a few cells from each subject, 10–30 cells per new born by Patil *et al.* (1972), 14 cells per infant by Bochkov *et al.* (1974) and 36 cells per infant by Hatcher and Hook (1981). The values reported in the present study are lower compared to the reported values so far, although protocol differences is a limiting factor in direct comparison.

There are only few reports on chromatid aberration frequencies in human populations, and often when reported the different types are lumped together sometimes as cells with aberrations. Galloway *et al.* (1986) and Bender *et al.* (1988) have published data on chromatid aberration frequencies in 41, 282 cells from 304 control subjects and 71, 950 cells from 341 cases respectively. They reported the frequencies of chromatid deletions 0.64% (range 0–6) and 0.809 (range 0–6) respectively.

Our data on SCEs in new borns is in agreement with that of Ardito *et al.* (1980) and Seshadri *et al.* (1982) who found a considerably lower SCE rate in cord blood than in mothers blood. The SCE rate measured from cord bloods in ten neonates was found to be significantly lesser than that of adults. A number of factors have been reported to influence the frequencies of SCEs in human peripheral blood lymphocytes, especially cigarette smoking. In our adult samples none were smokers. Das *et al.* (1986) however observed a higher frequency of SCEs 8.97/cell in cord bloods compared to 5.01/cell in 1–5 year infants. The authors explained the increased frequency of SCEs on the basis of variation in general hormonal status of the individual which can influence the formation of SCEs.

The background frequency of micronuclei in cord blood samples in 96 hr cultures was found to be between 2–5 per 1000 cells. The only other study on cord bloods (Aghamohammadi *et al.* 1984) reported a micronuclei frequency of 0.5–9.5 per 1000 cells with a modal value of 2.5. A wide range in the frequencies of spontaneous micronuclei in adult lymphocytes reported perhaps is due to the different methodologies, incubation time and scoring criteria then prevalent in the conventional micronucleus assay. The recent cytokinesis blocked micronucleus assay by Cytochalasin B may provide better insight in this aspect.

The implication of folate deficiency as a cause of *in vivo* chromosomal damage is supported by several lines of evidence. (Heath 1966, Menzies *et al.* 1966, Krogh Jensen 1977, Jackey *et al.* 1988, Li *et al.* 1986, Everson *et al.* 1988). Many women are given oral folic acid during pregnancy. Blood folate levels in the neonatal period are about twice as high as they are after 6–8 weeks of age (Matoth *et al.* 1964). The low incidence of spontaneous chromosome abberations, SCEs and micronuclei reported in the present study may possibly reflect the influence of high folate levels in cord bloods on spontaneous chromosomal damage.

The results obtained will provide baseline data for different studies and accurate baseline data on spontaneous chromosomal abberrations, SCEs and micronuclei, which are senstitive cytogenetic end points in the assessment of genetic damage, are important in monitoring human popualtion exposures to low levels of occupational and environmental genotoxicants.

Summary

A cytogenetic monitoring programme on human new borns carried out from 1983–1987 to find out the incidence of constitutional chromosome anomalies also included studies to obtain baseline data on spontaneous chromosomal aberrations (CA), sister chromatid exchanges (SCEs) and micronuclei (MN) from cord blood samples in limited number of human neonates. The results are compared with data obtained in our laboratory on these cytogenetic end points from adult peripheral blood lymphocytes. The micronuclei data was obtained from adult lymphocytes by the cytokinesis blocked lymphocyte assay, by Cytochalasin B. 120 cord blood samples analysed gave a mean frequency of 0.0052 chromatid breaks/metaphase and 0.0059 chromatid gaps/metaphase. Only one dicentric chromosome was noticed in the

12,000 cells analysed. No statistically significant difference was noticed between male and female new borns in the spontaneous CA frequency. 20 adult blood samples analysed after 48 hr culture and 10 adult blood samples analysed after 72 hr culture gave mean frequency of 0.0085, 0.013 chromatid breaks/metaphase and 0.01414, 0.019 chromatid gaps/metaphase respectively. A large statistically significant elevation in SCE/cell was found when adult blood samples were compared to cord blood samples (the difference 1.94 SCE/cell, $P<0.001$ by simple 't' test) the mean frequency of SCEs observed in cord blood samples and adult samples being 5.56 ± 0.21 and 7.496 ± 0.21 SCE/cell respectively. The frequency of micronuclei in cord blood lymphocytes was found to be 0–1.5/1000 cells, 1–3/1000 cells, 2–5/1000 cells in 48, 72 and 96 hr cultures respectively. Adult blood lymphocytes exhibited a micronuclei frequency of 0.0123 ± 0.001 MN/CB cell. The low incidence reported here of all three cytogenetic end points may possibly reflect the influence of high folate levels in cord bloods on spontaneous chromosomal damage.

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