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Micronucleated erythrocytes in preterm newborns in relation to maternal pathology.

Original Article

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SUMMARY.

Introduction. The micronucleus assay detects DNA damage. Certain pathologies that are associated with free radical production may increase the frequency of micronucleated erythrocytes (MNE). Here, we have investigated whether pathologies that are associated with the formation of free radicals lead to an increase in embryonic MNE frequencies during pregnancy.

Materials and Methods. We have used the in vivo micronuclei test on peripheral blood erythrocytes to determine MNE frequencies in preterm newborns (PN) from apparently healthy mothers. MNE frequencies were compared with those obtained from the peripheral blood from PN born to mothers with diabetes mellitus (DM), systemic arterial hypertension (SAH) or vaginal infection (VI). Blood samples were grouped according to gender, maternal pathology, and maternal folic acid intake.

Results. In the peripheral blood of PN born to mothers with pathologies the MNE and micronucleated polychromatic erythrocytes (MNPCE) frequencies were higher than in the PN born to mothers without any pathological condition ($P<0.02$). In the PN born to mothers with DM both the MNE and MNPCE frequencies were elevated ($P<0.01$), whereas in the PN born to mothers with VI only the MNE frequency was elevated ($P<0.03$). The MNE frequency in PN whose mothers suffered SAH showed an increase, although the MNE frequency was reduced in female PN and in PN whose mothers took folic acid ($P>0.05$). The results were evaluated by means of a U-Mann Whitney test.

Discussion. Our results show that the frequency of MNE increased in PN born to mothers with pathologies that involved an increase in free radical production. ***(Rev Biomed 2006; 17:11-16)***

Key words: micronucleated erythrocytes; preterm newborns; maternal pathology; free radicals; teratogenic potential.

RESUMEN.

Eritrocitos micronucleados en recién nacidos pretérmino en relación con la patología materna.

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Introducción. La prueba de micronúcleos detecta daño al ADN. Algunas patologías incrementan la frecuencia de eritrocitos micronucleados (EMN) debido a la producción de radicales libres asociados con el proceso de la enfermedad. En el presente trabajo, investigamos si la frecuencia de EMN en el embrión se incrementa por patologías que ocurren durante el embarazo y que involucran la formación de radicales libres.

Material y métodos. Mediante la prueba de micronúcleos (MN) in vivo en eritrocitos de sangre periférica, las frecuencias de EMN en recién nacidos pretérmino (RNP) de madres sin patología fueron comparadas con RNP de madres con diabetes mellitus (DM), hipertensión arterial sistémica (HAS) o infección vaginal (IV). Las muestras se agruparon respecto al género, patología materna e ingesta materna de ácido fólico.

Resultados. Las frecuencias de EMN y eritrocitos policromáticos micronucleados (EPCMN) en RNP de madres con patología fueron mayores que los de madres sin patología ($P<0.02$). RNP de madres con DM tuvieron frecuencia elevada de EMN y EPCMN ($P<0.01$), mientras que los EMN incrementaron ($P<0.03$) en los RNP de madres con IV. Los EMN en RNP de madres con HAS se incrementaron y se observó disminución en niñas RNP y en RNP cuyas madres recibieron ácido fólico ($P>0.05$). Los resultados fueron evaluados por medio de la prueba U-Mann Whitney.

Discusión. Nuestros resultados muestran aumento en los EMN de RNP de madres con patologías que involucran incremento en la producción de radicales libres. ***(Rev Biomed 2006; 17:11-16)***

Palabras clave: eritrocitos micronucleados; recién nacidos pretérmino; patología materna; radicales libres; potencial teratógeno.

INTRODUCTION.

The assay of peripheral blood micronucleated erythrocytes (MNE) is a simple, rapid and relatively inexpensive test to determine DNA damage (1-2).The assay is conducted in tissues with a high degree of cellular proliferation (3-4) and the results of the test can be interpreted in a straightforward manner. Hence, this test is a useful way to detect DNA damage produced in vivo and it can be carried out in just a drop of blood.

Micronuclei (MN) are chromosome fragments or whole chromosomes that are left behind in the cytoplasm during mitosis (1, 5-6). In some species, it is possible to measure the spontaneous formation of MN in peripheral blood (7-9), and an increase in MN is normally observed when organisms are exposed to genotoxic agents (10-11). Organisms with high levels of spontaneous MNE formation generally tolerate their existence in the peripheral circulation and, a chronic increase in the production of MN can result in the accumulation of MNE (10-11). However, in both young and adult humans the basal MNE frequencies in peripheral blood are close to zero. Indeed, due to the efficiency of the spleen at removing circulating MNE, not even exposure to micronucleogenic drugs increases the frequency of MNE in humans (6-7,12). As a result, MNE can typically only be observed in humans who have spleen dysfunction (1,6,13-14) or have been splenectomized (15-16). In contrast, the immature reticuloendothelial system of Preterm Newborns (PN) does not efficiently remove MNE from circulation and it is possible to observe MNE in the peripheral blood of PN (9).

There is an increase in the incidence of embryonic malformations and neonatal death when pregnant women suffer the effects of pathologies involving increased free radical production (17). From studies with experimental animals, it has been suggested that these congenital malformations may result from the alteration of serum factors and that they are associated with pathologies in the intrauterine environment (18).

Some pathological conditions associated with increases in free radical production have been related to DNA damage in peripheral blood lymphocytes (19). The DNA damage produced by free radicals results from their ability to cause breaks in the DNA chain and deoxyribose degradation (20-

23). These observations raise the question as to whether the diverse pathologies that involve the production of free radicals during pregnancy increase the formation of MN and thus, damage the fetus. Previously, lymphocyte culture from umbilical cord samples and micronucleus evaluation had been used to identify the effects of transplacental mutagens (24). In the present study we have investigated whether pathological conditions during pregnancy that are associated with increased free radical production can affect MNE frequencies in PN.

MATERIAL AND METHODS.

Blood samples were obtained from 78 PN born between 24 to 36 weeks of gestation at the Intensive Care section of the Neonatal Unit, Pediatric Service of the "Hospital Civil Juan I Menchaca", Universidad de Guadalajara. Each sample consisted of a few drops of blood from routine hospital blood samples. Information regarding the mother's health status, the drugs administered during pregnancy, the administration of folic acid supplements during the first three months of pregnancy, the weeks of gestation and newborn gender were obtained from the hospital records. PN samples were grouped by maternal pathology as follows: one group where there were no known pathologies, considered as without pathology (WP, n=37); a group with vaginal infection (VI, n=24); a group with systemic arterial hypertension (SAH, n=10); and a group with diabetes mellitus (DM, n=7).

Sample preparation and MNE analysis. Two smears were made from the drops of PN blood on clean coded slides. The slides were air-dried, fixed in absolute ethanol for 10 min, stained with acridine orange (25) and them examined using an Olympus CX31 fluorescence microscope. For each sample, the number of MNE in 10,000 total erythrocytes (TE), the number of micronucleated polychromatic erythrocytes (MNPCE) in 1,000 polychromatic erythrocytes (PCE), and the number of PCE in 1,000 TE was recorded.

Statistical analysis. Data are presented as the mean ± standard deviation. The results were evaluated using the Statistical Program for Social Sciences (SPSS v11.0) for Windows® medical pack (SPSS, Chicago Ill) applying a U-Mann Whitney test. A P-value of less than 0.05 was considered significant.

RESULTS.

The overall frequencies of MNE/10,000 TE, MNPCE/1,000 PCE, and PCE/1,000 TE in the 78 PN samples were 4.3±4.4, 1.0±1.5, and 34.2±26.5, respectively. No relationship was observed between the weeks of gestation and the frequency of MNE.

There were no significant differences detected between male and female newborns in terms of the MNE and MNPCE frequencies, irrespective of the maternal health status during pregnancy (P>0.05, Table 1). In addition, the overall MNE and MNPCE frequencies in PN born to mothers who had suffered pathological conditions during pregnancy were higher than the frequencies for PN born to the WP mothers (Table 2). Indeed, increases were detected in both MNE and MNPCE frequencies from PN born to mothers affected by VI, SAH, or DM during pregnancy (Table 2). However, only the increase in MNE for PN born

Table 1
Effect of PN gender on the frequencies of MNE, MNPCE and PCE.

	Females n=27	Males n=48	Significance
MNE/10,000 TE	3.8±3.8	4.7±4.8	NS
MNPCE/1,000 PCE	1.0±1.3	1.1±1.6	NS
PCE/1,000 TE	39.0±27.1	29.9±24.5	NS

Data are expressed as mean ± standard deviation. PN: Premature newborns; NS: not significant; n: sample size. MNE: Micronucleated erythrocytes; MNPCE: Micronucleated polychromatic erythrocytes; PCE: Polychromatic erythrocytes; TE: Total erythrocytes.

Table 2
Effect of maternal VI, SAH, and DM during pregnancy on the frequencies of MNE, MNPCE and PCE in PN.

	Without pathology n=37	With pathology n=41	VI n=24	SAH n=10	DM n=7
MNE/10,000 TE	3.5±4.1	5.1±4.6	4.7±3.2	4.6±6.9	7.7±4.8
		P<0.02	P<0.03	NS	P<0.01
MNPCE/1,000 PCE	0.7±1.4	1.3±1.6	1.1±1.3	1.5±2.2	2.0±1.8
		P<0.02	NS	NS	P<0.01
PCE/1,000 TE	34.4±27.7	34.0±25.8	31.8±27.3	20.8±14.9	60.4±11.2
		NS	NS	NS	P<0.01

Data are expressed as mean ± standard deviation. VI: Vaginal infection; SAH: Systemic arterial hypertension; DM: Diabetes mellitus; MNE: Micronucleated erythrocytes; MNPCE: Micronucleated polychromatic erythrocytes; PCE: Polychromatic erythrocytes; TE: Total erythrocytes; PN: Preterm newborns; NS: not significant; n: sample size. All comparisons were made against group without pathologies.

to mothers who had VI or DM were statistically significant (P<0.03). Interestingly, no significant differences were observed in MNE frequencies in PN born to mothers who took folic acid supplements during pregnancy (P>0.05, Table 3).

DISCUSSION.

The presence of MNE in PN peripheral blood can be used to evaluate the teratogenic potential of drugs administered during pregnancy and the effect of maternal pathology in PN as was demonstrated in an experimental model (26). In a previous work, the micronucleus frequency in lymphocyte cultures from human umbilical cord samples was shown to possibly be useful to identify transplacental mutagens (24). In the present study we evaluated the possibility of using a direct method that does not require cell culture and that can be performed on PN, to asses the effects of maternal pathological conditions or the drugs taken during pregnancy on the PN. We found that PN born to mothers with DM had significantly higher frequencies of MNE than PN born to mothers who did not suffer any pathology during pregnancy. These results agree with those described by Levario-Carrillo *et al*., (2005), who showed that the number of micronucleated lymphocytes increased in newborns from mothers with a complicated course of pregnancy and that there was no significant difference between genders. Among the pathologies that were studied, DM was the one associated with the highest number of MN. In this pathology, a cause effect relationship between the free radicals produced and the incidence of embryonic malformation is known (17). Hence, the increased MNE frequency that we detected may be a result of the accumulated DNA damage produced by the free radicals generated as a result of DM.

Others pathologies like SAH increase the

Table 3
Effect of maternal folic acid supplementation during pregnancy on the frequency of MNE, MNPCE and PCE in PN.

	With folic acid n=12	Without folic acid n=66
MNE/10,000 TE	2.8±2.6	4.6±4.6
		NS
MNPCE/1,000 PCE	0.7±1.0	1.1±1.6
		NS
PCE/1,000 TE	43.9±36.0	32.4±24.4
		NS

Data are expressed as mean ± standard deviation. NS: not significant; n: sample size. MNE: Micronucleated erythrocytes; MNPCE: Micronucleated polychromatic erythrocytes; PCE: Polychromatic erythrocytes; TE: Total erythrocytes; PN: Preterm newborns.

production of free radicals and, as a consequence, they might increase MN frequency, as occurs in conditions that involve an inflammatory processes (19,27). It is known that nitric oxide (NO) is produced by the immune system in infectious diseases (28) such as VI, and this could explain the significant increase in the MNE frequency in PN born to mothers diagnosed with this condition.

It is has been reported that low folate levels can also give rise to an increase in MNE frequencies (29). Since the administration of a folic acid supplement to pregnant mothers is thought to prevent cranial neural tube defects (30-31), pregnant women who receive folic acid during pregnancy might also have lower MNE frequencies. While the MNE and MNPCE frequencies in PN from mothers receiving folate supplements were reduced, this effect was modest and the sample was not large enough to demonstrate a significant effect.

A significant effect on the MNE or MNPCE frequencies was detected in PN born to mothers with DM. It is possible that increasing the sample size for the other experimental groups considered might be helpful in demonstrating a relationship between the specific pathology and MNE accumulation. Furthermore, obtaining blood samples as soon as possible after birth might aid in obtaining more homogeneous MNE frequencies. This variable was not controlled in the present study. The efficiency of the spleen in removing the MNE from circulation and the kinetics of MNE persistence are parameters that are not well defined in human PN. Nevertheless, the results obtained here do suggest that the increase in the MNE frequency in PN might be related to maternal pathologies during pregnancy which produce an increase in free radical production.

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