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INFLUENCE OF DEMOGRAPHIC PARAMETERS AND DIETARY HABITS ON THE CYTOME ASSAY BIOMARKERS IN LYMPHOCYTES AND BUCCAL EPITHELIAL CELLS FROM A GROUP OF ARGENTINE ADOLESCENTS

INFLUENCIA DE PARÁMETROS DEMOGRÁFICOS Y HÁBITOS ALIMENTARIOS SOBRE BIOMARCADORES DEL ENSAYO CITOMA EN LINFOCITOS Y CÉLULAS EPITELIALES BUCALES DE ADOLESCENTES ARGENTINOS

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ABSTRACT

The use of the cytome assay in monitoring studies on children has increased in recent years. For this reason, it is necessary to know the role of possible confounding factors that could affect its outcomes. The objective was to evaluate the influence of some demographic variables and diet on the baseline values of the cytome assay biomarkers in lymphocytes and buccal mucosa cells from a group of Argentine adolescents. Following the calculation of the biomarkers, a multivariate regression analysis including confounders was performed. In lymphocytes it was observed that micronuclei (MNi) had a negative association with a moderate consumption of roots and tubers, while the number of nuclear buds (NBUDs) was higher in minors not exposed to second-hand smoke (SHS). Regarding epithelial cells, MNi had a negative relationship with the intake of tropical fruits and red meat; on the contrary, this parameter increased with the moderate ingestion of legumes. In addition, oral NBUDs had a positive association with citrus and red meat consumption, whereas cereals and oil decreased its frequency. Furthermore, an increased number of binucleated cells was observed for adolescents who ate white meat and an increase in pyknotic cells for those exposed to SHS. These results revealed that in adolescents the baseline level of the cytome assay biomarkers, especially of those related to genotoxicity, can be influenced by exogenous variables, for instance, dietary habits. Thus, such factors need to be considered when carrying out biomonitoring studies on child populations.

Key words: baseline values, CBMN-cyt, confounding factors, individual food preferences, young population

RESUMEN

El uso del ensayo de citoma en estudios de seguimiento en niños se ha incrementado en los últimos años y resulta necesario conocer el papel de posibles factores de confusión que podrían afectar sus resultados. El objetivo fue evaluar la influencia de algunas variables demográficas y de la dieta en los valores basales de los biomarcadores de este ensayo en linfocitos y células de mucosa bucal de un grupo de adolescentes argentinos. Luego del cálculo de los biomarcadores, se realizó un análisis de regresión multivariada incluyendo factores de confusión. En linfocitos se observó que los micronúcleos (MN) se asociaron negativamente con un consumo moderado de raíces y tubérculos, mientras que los brotes nucleares (BrN) aumentaron en los menores no expuestos al humo de segunda mano (HSM). En células epiteliales, los MN tuvieron una relación negativa con el consumo de frutas tropicales y carnes rojas, aunque aumentaron con el consumo moderado de legumbres. Los BrN orales tuvieron una asociación positiva con el consumo de cítricos y carnes rojas, mientras que los cereales y el aceite disminuyeron su frecuencia. Además, se encontró un mayor número de células binucleadas para quienes comieron carne blanca y un aumento de células picnóticas para los expuestos a HSM. Estos resultados revelaron que en los adolescentes el nivel basal de los biomarcadores del ensayo de citoma, especialmente de aquellos relacionados con la genotoxicidad, puede verse influenciado por variables exógenas como los hábitos alimentarios. Por lo tanto, dichos factores deben considerarse al realizar estudios de biomonitoreo en poblaciones infantiles.

Palabras clave: valores basales, MNCB-cit, factores de confusión, preferencias alimentarias individual, población joven.

INTRODUCTION

In recent years, the number of human biomonitoring studies has risen following a substantial increase of interest in different environmental problems that affect people's health (WHO, 2015; Louro *et al.*, 2019). Within this group of studies those related to the child and adolescent population have gained importance since minors are more susceptible than adults to pollutants (Perlroth and Branco, 2017). In addition, there is evidence that associates early exposure to xenobiotics with the development of various chronic diseases in adulthood, including some related to genetic instability such as cancer (Landrigan *et al.*, 2004; WHO, 2011; Sly *et al.*, 2014).

Taking into account the relationship between environmental exposure to xenobiotics and its effect on DNA, different tests have been developed which measure biomarkers associated with this outcome (Beedanagari, 2014). One of the most popular approaches is the micronucleus test, especially the more comprehensive version known as cytome assay. This method evaluates a variety of biomarkers related to genotoxicity, chromosomal instability and cell death depending on the matrix in which it is carried out (Fenech, 2006; Sommer *et al.*, 2020).

The assay on a culture of lymphocytes with blocked cytokinesis, for example, evaluates micronuclei (MNi), nuclear buds (NBUDs) and nucleoplasmic bridges (NPBs). MNi are the result of chromosome fragments or whole chromosomes that are left behind during cell division, whereas NBUDs are related to the elimination of amplified DNA and/or DNA repair complexes, and NPBs are caused by dicentric chromosomes (telomere fusion) or by sister chromatids that cannot be separated due to defects in cohesins and/or separases (Fenech *et al.*, 2016). On the other hand, in buccal cells, apart from MNi and NBUDs, it is also possible to measure the frequency of binucleated (BN) cells as a result of cytokinesis failures and cells with nuclear anomalies such as condensed chromatin (CC), karyorrhexis (KHC), karyolysis (KYL) and pyknosis (PYK), which are associated with cell death processes (Thomas *et al.*, 2009).

Most studies in genetic toxicology report the use of the cytome assay in lymphocytes and buccal mucosa cells (BMC), with the cytokinesis-block micronucleus cytome (CBMN) assay in lymphocyte cultures being the version most used in genotoxicity assays and biomonitoring studies (Kirsch-Volders *et al.*, 2018; Nersesyan *et al.*, 2016). Indeed, understanding the molecular bases for formation of the biomarkers assessed by this approach have turned it into a robust test to such an extent that the frequency of micronuclei in this type of cells is considered as a predictor of cancer risk (Bonassi *et al.*, 2011b; Fenech *et al.*, 2016). On the other hand, the use of the cytome assay in BMC has recently increased, mainly due to the methodological advantages it provides. Firstly, sampling for this test is minimally invasive, which is ideal for working with children and people reluctant to venipuncture. Furthermore, this assay does not require cell culture, so it is easily carried out in laboratories without the necessary equipment for cell maintenance (Thomas *et al.*, 2009; Holland *et al.*, 2008).

Different observational studies on children and adolescents have used the cytome assay in lymphocytes or BMC in order to assess the effect of exposure to environmental pollutants (Neri *et al.*, 2006; Castañeda-Yslas *et al.*, 2016; Martínez-Perafán *et al.*, 2018; Lemos *et al.*, 2020; Panico *et al.*, 2020), and many others report the basal frequency of the biomarkers of this test in healthy young people (Neri *et al.*, 2005; Gajski *et al.*, 2013; Silva da Silva *et al.*, 2015). Following this, the relevance of the cytome assay in the biomonitoring of child population is evident; however, there is still a lack of information about the influence that some demographic, genetic and lifestyle factors could have on the levels of the biomarkers measured, especially in some of those evaluated in the buccal test (Bonassi *et al.*, 2011b). Indeed, some characteristics such as age, sex and diet could be confounding factors or moderating variables, which has already been analysed in the lymphocyte test (Fenech and Bonassi, 2011; Holland *et al.*, 2011). Therefore, it is crucial to carry out specific studies with a biomonitoring that evaluates the association between these factors and the frequency of the cytome assay markers in a multivariate model.

Following the aforementioned, this study had two objectives: first, to determine the baseline levels of the biomarkers of the CBMN assay in lymphocytes and of the buccal micronucleus cytome (BMCyt) assay in a group of Argentine adolescents not environmentally exposed to chemical agents except for second-hand smoke (SHS). Second, to evaluate the possible association between the main demographic variables of the group and their dietary habits, and the levels of the biomarkers of both tests in a multivariate model.

MATERIALS AND METHODS

Study population and ethical considerations

The present study is a descriptive cross-sectional analysis carried out in a group of 54 school-age adolescents of both sexes. All participants live in Exaltación de la Cruz, a rural zone in the Province of Buenos Aires, Argentina. This community is part of a social integration program at the University of Buenos Aires. The adolescents' parents or legal guardians signed the corresponding informed consent, and were later interviewed in order to fill an anamnesis survey

and dietary questionnaire. Both the surveys and the informed consent followed the guidelines established in the Declaration of Helsinki (WMA, 2013). Furthermore, the study was approved by the Ethics Committee of the Faculty of Pharmacy and Biochemistry of the University of Buenos Aires (EXP-FYB N° 0087945/2016).

Adolescents with any severe disease or familiar cancer history were not part of this study. Additionally, adolescents exposed to environmental or therapeutic xenobiotics such as antibiotics and other medication, as well as those who had undergone radiation therapy or X-rays six months prior the sample collection, were excluded.

Food consumption frequency questionnaire (FFQ)

Taking into account the demographic characteristics of the population and their possible dietary habits, a questionnaire constructed by the authors and previously tested with people from the same region was used (Martínez-Perafán *et al.*, 2018). The survey collected information about the consumption per week of a list of foods from the traditional Argentine diet; these foods were later sorted into groups based on their nutritional characteristics. In this way, 13 food groups were formed: citrus, tropical fruits, fruit vegetables, leaf vegetables, roots and tubers, legumes, dairy products and eggs, red meat, white meat, lunch meat, cereals, fat and oil, and candies. Finally, a frequency-variety index was calculated for each group by multiplying the average frequency of consumption per week of all foods within the same group by the number of foods that had a frequency of consumption greater than or equal to once per week.

Collection of biological samples

Peripheral blood samples (n=43) were taken by venipuncture and collected in heparinised tubes, while buccal epithelium samples (n=54) were obtained by scraping the inside of each cheek with different cytological brushes, which were later put into a centrifuge tube containing Saccomannos' fixative (Biopur, Rosario, Argentina). All samples were taken in the morning, then they were stored in ice, protected from light and transported to the laboratory. Blood samples were usually processed two hours after taking the last sample, whereas those from buccal mucosa were stored at 4 °C until processing (no more than two weeks after sampling).

Cytokinesis-block micronucleus cytome (CBMN) assay

The CBMN assay in lymphocytes was performed following the indications proposed by Fenech (2007) with minimal modifications. According to this, lymphocytes were isolated from the peripheral blood samples using Ficoll-Paque (GE Healthcare Bio-Sciences, Uppsala, Sweden). Subsequently, 106 cells/well were cultivated in a 12-well culture plate with 1 mL/well of RPMI 1640 medium (Gibco®, Life Technologies Corporation, New York, US) supplemented with fetal bovine serum (15%; Internegocios, Buenos Aires, Argentina) and phytohemagglutinin (10 µg/mL; Gibco®, Life Technologies Corporation, New York, US). Cultures were incubated at 37 °C and after 44 h, cytochalasin B (4.5 µg/mL; Sigma-Aldrich, Steinheim, Germany) was added to each well in order to block cytokinesis. Later, 72 h after the establishment of the cultures, the lymphocytes were harvested, placed onto a slide and fixed with methanol (Merck, Darmstadt, Germany). Finally, the cells were stained with Giemsa (10%; Merck, Darmstadt, Germany) and 2000 BN cells were scored per individual at 1000x magnification, using a transmitted light microscope (Olympus CX31). The frequencies of the genotoxicity biomarkers MNi, NBUDs and NPBs were reported.

Buccal micronucleus cytome (BMCyt) assay

This test was carried out following the protocol of Thomas *et al.* (2009) with some adjustments. Firstly, buccal mucosa samples were washed three times with a buffer solution (0.1 M EDTA, Sigma-Aldrich, Steinheim, Germany; 0.01 M Tris–HCl, Sigma-Aldrich, Steinheim, Germany, and 0.02 M NaCl, Merck, Darmstadt, Germany). In order to achieve optimal cell disaggregation, 50 µL of DMSO (Merck, Darmstadt, Germany) per mL of cell suspension was added during the first two washes. After the third wash, the cells were transferred onto a slide, fixed with methanol (Merck, Darmstadt, Germany) and stained with propidium iodide (30 µg/mL; Sigma-Aldrich, Steinheim, Germany). Finally, 2000 cells per individual were scored by a fluorescence microscope at 400x magnification (Olympus BX-40F4). The frequencies of MNi and NBUDs were considered as genotoxicity biomarkers, whereas, CC, KHC, PYK and KYL were reported as cell death parameters, and BN as a result of failures in cytokinesis.

Statistical analysis

The data analysis consisted of two parts, first it was performed a univariate analysis of the demographic characteristics of the adolescents, their dietary information and the frequency of biomarkers in both lymphocytes and BMC. Secondly, a multivariate regression analysis between each cytogenetic biomarker and the main demographic characteristics of the adolescents was carried out. Similarly, a multivariate test was applied to find out the relationship between the biomarkers and the dietary habits of the study participants.

A negative binomial distribution was used for the multivariate analysis, this approach is especially effective with count data such as those obtained from the scoring of the cytome assay biomarkers (Ceppi *et al.*, 2010). Regarding the multivariate analysis with demographic characteristics, the previous step was to categorize the independent variables, avoiding categories with low frequencies when possible. The demographic characteristics taken into account were age, sex, and weight status (endogenous factors), and the exposure to SHS (exogenous variable).

On the other hand, prior to the multivariate analysis with the food groups, a bivariate analysis was carried out to select the variables that would fit in the model. Following this purpose, the values corresponding to the frequency-variety index of each food group were categorized into tertiles of the observed distribution, with the indices within the first tertile being the lowest, and those in the third tertile being the highest. In addition, the mean frequency of each biomarker in each of the three tertiles was calculated. Subsequently, the effect of consuming a certain food group was described as the percentage variation between the average frequency of each biomarker in the second and third tertiles compared to the mean of the first tertile. After the bivariate approach, the variables that presented a p-value less than 0.2 in relation to each biomarker were selected to be included in the multivariate regression model. Additionally, the variance inflation test was used as a second criterion for the exclusion of independent variables that presented multicollinearity.

Univariate analysis was carried out using IBM SPSS Statistics for Windows (IBM Corp, 2015), whereas negative binomial regression models, both bivariate and multivariate, were conducted in R (R Core Team, 2019) using the MASS (Venables and Ripley, 2002), jtools (Long, 2020) and car (Fox and Weisberg, 2019) packages.

Ethics approval and Informed consent

The present study was reviewed and approved by the Ethics Committee of the Faculty of Pharmacy and Biochemistry of the Universidad de Buenos Aires (EXP-FYB N° 0087945/2016), following national and international guidelines for research on humans.

This study was carried out with the written consent of the participants' parents or legal guardians.

RESULTS

Demographic characteristics and frequency of biomarkers

The main demographic characteristics and frequency of cytome assay biomarkers in peripheral blood lymphocytes (PBL) and BMC from the adolescents are listed in Table 1. The average age of the volunteers was 11.19 years (range from 10 to 14 years old). In addition, it was observed that more men than women participated in the study and that exposure to SHS was frequent. It should also be noted that there was no significant difference between the body mass index of male and female individuals (p=0.346), and most of the participants had a normal weight according to the criteria of the WHO (2009). Furthermore, considering the anamnesis data, it was also possible to verify that despite living in a rural area, the adolescents were not directly or indirectly exposed to agrochemicals or other types of environmental xenobiotics (data not shown), with the exception of cigarette smoke from parents who smoked.

In relation to the cytogenetic biomarkers, the CBMN assay revealed that the mean baseline frequencies of genotoxicity markers in the adolescent lymphocytes were low (in PBL MNi= 1.87‰, and in BMC MNi= 3.23‰ and NBUDs= 1.29‰). Similarly, the micronucleus cytome assay in BMC showed that the average of baseline values associated with genotoxic risk and cytokinesis failure were also close to zero, while the parameters related to cell death had a diverse mean frequency (Table 1).

Relationship between biomarkers in PBL and demographic characteristics

Table 2 shows the results of the multivariate analysis between the different biomarkers in PBL and the main demographic characteristics. There is a statistically significant relationship between exposure to SHS and NBUDs, with a significantly higher frequency in adolescents whose parents did not smoke (factor of 116%, p=0.028).

Relationship between biomarkers in BMC and demographic characteristics

A significant association (p=0.044) was observed between age and the frequency of NBUDs in BMC. Specifically, 11-year-old adolescents had a 51% decrease in this marker compared to 10-year-olds (Table 3). Similarly, Table 4 displays that the average number of PYK cells in the buccal mucosa was significantly lower in adolescents not exposed to SHS than in those exposed (percentage variation 57%, p=0.046).

Relationship between biomarkers in PBL and dietary habits

After the bivariate analysis between cytogenetic markers and different food groups (Table 5), a multivariate regression model was built to illustrate the relationship between biomarkers and dietary habits. Following this, Table 6 shows the results of the multivariate model between the genetic damage markers in lymphocytes and the short-listed food groups. Only a significant reduction (32%) in the frequency of MNi (p=0.032) was

Table 1. Mean value and standard error (SE) of demographic characteristics and frequencies of cytome assay biomarkers in peripheral blood lymphocytes (PBL) and buccal mucosa cells (BMC) in a group of Argentinean adolescents.

	n	Mean	SE
Age (years)	54	11.19	1.30
Sex			
Female	25	—	—
Male	29	—	—
BMI per sex*			
Female	25	20.98	1.15
Male	29	19.77	0.63
Weight status (♀:♂)			
Normal weight	35 (16:19)	—	—
Overweight	9 (4:5)	—	—
Obesity	10 (5:5)	—	—
Exposure to second-hand smoke			
Exposed	31	—	—
Unexposed	23	—	—
Biomarkers in PBL (‰)			
MNi	43	1.87	0.19
NBUDs	43	0.28	0.07
NPBs	43	0.15	0.04
Biomarkers in BMC (‰)			
MNi	54	3.23	0.77
NBUDs	54	1.29	0.25
BN	54	0.76	0.14
CC	54	45.44	4.53
KHC	54	15.07	1.30
PYK	54	0.61	0.14
KYL	54	19.89	2.49

*No significant difference between body mass index (BMI) averages of females and males (p=0.346).

MNi: micronuclei; NBUDs: nuclear buds; NPBs: nucleoplasmic bridges; BN: binucleated cells; CC: cells with condensed chromatin; KHC: karyorrhectic cells; PYK: pyknotic cells; KYL: karyolytic cells.

Table 2. Data obtained from a multivariate analysis between the different biomarkers in peripheral blood lymphocytes (PBL) and the main demographic characteristics

	MNi			NBUDs			NPBs		
	Percentage variation	95% CI	*p*	Percentage variation	95% CI	*p*	Percentage variation	95% CI	*p*
Age (years)									
10	0	—	—	0	—	—	0	—	—
11	-25.06	0.35/1.12	117	-44.19	0.10/1.05	61	-31.03	0.14/4.53	811
12	4.51	0.47/1.73	747	-41.86	0.07/1.27	102	89.66	0.56/21.19	183
13 - 14[a]	-24.83	0.27/1.63	372	-22.09	0.07/2.27	292			
Sex									
Female	0	—	—	0	—	—	0	—	—
Male	0.27	0.60/1.39	680	34.04	0.60/3.80	388	-50.00	0.09/1.20	92
Weight status									
Normal weight	0	—	—	0	—	—	0	—	—
Overweight	-21.53	0.47/1.64	690	-18.03	0.31/5.00	761	60.00	0.45/4.61	537
Obesity[b]	-20.30	0.40/1.19	185	-27.87	0.20/2.17	490			
SH smoke									
Exposed	0	—	—	0	—	—	0	—	—
Unexposed	6.30	0.72/1.64	696	115.79	1.12/7.62	0.028*	-6.45	0.14/2.11	373

Percentage variation and *p*-values refer to comparison with the first category of each variable.

[a] The last category for NPBs was formed with adolescents from 12 to 14 years old

[b] The last category for NPBs was formed with adolescents with overweight and obesity

* Significant difference

MNi: micronuclei; NBUDs: nuclear buds; NPBs: nucleoplasmic bridges; CI: confidence interval

found for young people whose intake of roots and tubers was located in the second tertile compared to those who presented a consumption belonging to the first tertile.

Relationship between biomarkers in BMC and dietary habits

The association between the consumption of different types of food and markers of genotoxicity and cytokinesis failure is shown in Table 7. It can be observed that the highest level of intake of tropical fruits and red meat was related to a decrease in the frequency of MNi (83%, p=0.007 and 69%, p=0.006, respectively) when compared to the first tertile of consumption. On the contrary, a higher intake of legumes (tertile 2) was associated with an increase of 133% in this biomarker (p=0.009); however, this effect was not significant for the third tertile of consumption. Furthermore, the highest ingestion of citrus and red meat had an influence on the rise in the number of NBUDs (122%, p=0.022 and 175%, p=0.006, respectively). While the frequency of this parameter fell with the intake of cereals both in tertile 2 and 3 (39%, p=0.007 and 49%, p=0.0002), this effect was similar with the consumption of fat and oil but only up to the second tertile (95%, p=0.011). In addition, an increase in BN cells associated with the highest level of white meat consumption was observed (126%, p=0.004). In contrast to the genotoxicity results of the BMCyt assay, the cell death biomarkers of this test showed no association with any of the food groups included in the multivariate model (Table 8).

DISCUSSION

This study shows the baseline values of the cytome assay biomarkers in two different cell types from Argentine adolescents (10–14 years old) and their association with possible confounding variables. With regard to the frequency of genotoxicity parameters in lymphocytes, it was found that values of MNi, NBUDs and NPBs were comparable with those described by Gajski *et al.* (2013)

Table 3. Data obtained from a multivariate analysis between demographic characteristics and baseline frequency of genotoxicity and cytokinesis failure biomarkers in buccal mucosa cells (BMC)

	MNi			NBUDs			BN		
	Percentage variation	95% CI	*p*	Percentage variation	95% CI	*p*	Percentage variation	95% CI	*p*
Age (years)									
10	0	—	—	0	—	—	0	—	—
11	-42.80	0.20/3.38	788	-51.22	0.09/0.97	0.044*	-35.00	0.20/1.83	371
12	45.20	0.35/9.99	466	-21.46	0.11/1.80	251	66.25	0.57/5.48	320
13 - 14	33.33	0.24/15.67	537	-57.32	0.04/1.62	147	56.25	0.48/7.08	377
Sex									
Female	0	—	—	0	—	—	0	—	—
Male	13.93	0.41/2.90	859	10.25	0.42/2.26	963	34.37	0.49/2.13	962
Weight status									
Normal weight	0	—	—	0	—	—	0	—	—
Overweight	-66.28	0.11/1.64	216	23.90	0.51/4.74	431	85.00	0.99/6.06	52
Obesity	20.12	0.31/4.00	879	-8.37	0.18/1.81	345	66.67	0.66/4.03	284
SH smoke									
Exposed	0	—	—	0	—	—	0	—	—
Unexposed	-11.57	0.24/1.82	426	8.87	0.64/3.59	350	-3.90	0.45/2.10	942

Percentage variation and *p*-values refer to comparison with the first category of each variable

* Significant difference

MNi: micronuclei; NBUDs: nuclear buds; BN: binucleated cells; CI: confidence interval

in their study on healthy Croatian children (4–14 years old). In addition, the average frequency of MNi found in the present study (1.87‰) was lower than that informed by Neri *et al.* (2005) for minors aged 10 to 14 years (6.02‰) in their pooled analysis. Furthermore, the levels of markers found in Argentine adolescents were similar to those reported for reference groups, or individuals not exposed, in other studies on child population (Kapka *et al.*, 2007; Milne *et al.*, 2015; Mørck *et al.*, 2016). Therefore, due to the similarities between the levels of the tested biomarkers and the results of other reports carried out on healthy children, it can be stated that the adolescents participating in the present study did not present genomic damage evaluated with the cytome assay in PBL.

Comparison of baseline levels of BMCyt assay parameters in the Argentine adolescents with those found in other studies revealed that the mean frequency of MNi and NBUDs were slightly higher in the Argentine minors than in healthy children selected as reference groups in biomonitoring studies from Italy (Villarini *et al.*, 2018; Panico *et al.*, 2020) Brazil (Sisenando *et al.*, 2012; Silva da Silva *et al.*, 2015), Mexico (Neri *et al.*, 2006; Gómez-Arroyo *et al.*, 2013) and Malaysia (Sopian *et al.*, 2020). Furthermore, the mean frequencies of the biomarkers of cell death and cytokinesis failure in the participants of the present study were similar to those of the reference group described by Villarini *et al.* (2018). In contrast, the mean frequency of PYK and KHC cells was higher in Brazilian children (Silva da Silva *et al.*, 2015), whereas the values of all markers of this type were much lower in the studies carried out on Mexican minors (Gómez-Arroyo *et al.*, 2013; Castañeda-Yslas *et al.*, 2016). It was also observed that some studies only mentioned the frequencies of parameters related to genotoxic risk (Sisenando *et al.*, 2012; Panico *et al.*, 2020; Sopian *et al.*, 2020). Despite the differences between the reports, it should be noted that the values of the biomarkers in this study were within the ranges proposed by Thomas *et al.* (2009) in healthy young people, except for the frequency of KYL cells, which was lower, as in most of the other publications.

Table 4. Data obtained from a multivariate analysis of demographic characteristics of the adolescents and the baseline frequency of cell death biomarkers in buccal mucosa cells (BMC)

	CC			KHC			PYK			KYL		
	Percentage variation	95% CI	p	Percentage variation	95% CI	p	Percentage variation	95% CI	p	Percentage variation	95% CI	p
Age (years)												
10	0	—	—	0	—	—	0	—	—	0	—	—
11	47.43	0.82/2.38	213	04.06	0.54/1.50	694	16.00	0.56/10.28	242	-6.72	0.35/1.63	466
12	98.90	0.89/3.16	107	-14.00	0.40/1.37	341	100.00	1.00/26.93	51	10.59	0.28/1.80	477
13 - 14	105.00	0.83/3.98	137	-37.07	0.27/1.30	188	-50.00	0.13/18.33	743	-5.64	0.18/1.87	368
Sex												
Female	0	—	—	0	—	—	0	—	—	0	—	—
Male	34.17	0.88/1.81	211	-7.63	0.69/1.39	889	-7.81	0.31/2.10	650	32.25	0.84/2.44	185
Weight status												
Normal weight	0	—	—	0	—	—	0	—	—	0	—	—
Overweight	1.27	0.64/1.68	889	4.38	0.62/1.58	963	-6.67	0.21/2.80	686	-30.03	0.39/1.62	522
Obesity	-30.50	0.50/1.32	399	-20.21	0.43/1.12	132	16.67	0.59/6.45	275	-40.11	0.30/1.24	169
SH smoke												
Exposed	0	—	—	0	—	—	0	—	—	0	—	—
Unexposed	10.22	0.68/1.44	956	-14.04	0.60/1.24	426	-56.79	0.12/0.98	0.046*	28.41	0.75/2.25	354

Percentage variation and *p*-values refer to comparison with the first category of each variable

* Significant difference

CC: cells with condensed chromatin; KHC: karyorrhectic cells; PYK: pyknotic cells; KYL: karyolytic cells; CI: confidence interval.

The baseline level of the biomarkers evaluated by the cytome assay in both lymphocytes and BMC can vary between people according to some sociodemographic or lifestyle factors. For example, among the adult population, it has been observed that older people and women have higher frequencies of MNi in lymphocytes (Fenech and Bonassi, 2011). However, the multivariate model fitted in the present study showed no association between the genotoxicity biomarkers of the lymphocyte cytome assay and age or sex. These results can be attributed to the fact that the influence of these variables is not the same in the child population, in which the relationship between sex and the frequency of MNi in blood cells appears to be non-existent. Meanwhile, the effect of age would only be noticeable among minors with a considerable difference in age (Holland *et al.*, 2011).

The weight status of the participants showed no effect on the measured biomarkers in lymphocytes, which contrasts the results from Scarpato *et al.* (2011), who found that the number of MNi in children with overweight and obesity was significantly higher than in those with normal weight; however, the mean frequency of MNi in the three groups was relatively low, as they mentioned (<3‰). Other studies on adults found higher levels of these biomarkers in people with obesity and metabolic syndrome (Donmez-Altuntas *et al.*, 2014; Karaman *et al.*, 2015). Nevertheless, prospective studies are necessary, especially on children and adolescents, to establish if the DNA damage increase could be related to overnutrition or sedentary lifestyle. In this way, it could be determined whether the cytome assay outcomes are markers or mediators of health problems such as obesity and metabolic syndrome (Andreassi *et al.*, 2011).

On the other hand, no exposure to SHS was associated with a higher frequency of NBUDs. This result was unexpected since tobacco smoke is considered a genotoxic agent (DeMarini, 2004). However, a pooled analysis by Bonassi *et al.* (2003) including more than 5,710 participants showed a decrease in the MNi value of smokers and former smokers compared to non-smokers, what indicates that there does not appear to be a strong positive effect of smoking on the markers of the cytome test in PBL. In relation to exposure to indoor environmental tobacco smoke in children, only one study was found that reported an increase in

Table 5. Data obtained from a bivariate analysis between cytogenetic markers and different food groups in peripheral blood lymphocytes (PBL) and buccal mucosa cells (BMC)

	PBL			BMC						
	MNi	**NBUDs**	**NPBs**	**MNi**	**NBUDs**	**BN**	**CC**	**KHC**	**PYK**	**KYL**
Citrus										
Tertile 1	—	—	—	—	—	—	—	—	—	—
Tertile 2	948	928	645	258	65	82	916	379	506	975
Tertile 3	912	817	612	142	104	709	248	766	607	786
Tropical fruits										
Tertile 1	—	—	—	—	—	—	—	—	—	—
Tertile 2	585	803	118	793	710	962	859	162	234	324
Tertile 3	536	525	372	0.001*	200	759	383	776	710	310
Fruit vegetables										
Tertile 1	—	—	—	—	—	—	—	—	—	—
Tertile 2	409	912	775	837	99	449	420	105	50	614
Tertile 3	769	182	395	833	0.045*	482	277	742	277	972
Leaf vegetables										
Tertile 1	—	—	—	—	—	—	—	—	—	—
Tertile 2	354	0.046*	301	0.046*	488	773	681	684	414	666
Tertile 3	402	70	1.000	499	255	336	886	391	169	151
Roots and Tubers										
Tertile 1	—	—	—	—	—	—	—	—	—	—
Tertile 2	125	683	151	968	319	362	739	276	97	990
Tertile 3	867	281	591	251	672	884	373	612	681	878
Legumes										
Tertile 1	—	—	—	—	—	—	—	—	—	—
Tertile 2	199	376	158	166	413	898	0.008*	215	947	508
Tertile 3	725	440	86	316	367	723	772	760	373	303
Dairy products and eggs										
Tertile 1	—	—	—	—	—	—	—	—	—	—
Tertile 2	515	966	540	455	485	774	749	279	615	646
Tertile 3	138	320	306	583	804	636	314	203	663	605
Red meat[a]										
Tertile 1	—	—	—	—	—	—	—	—	—	—
Tertile 2	0.034*	489	427	876	885	56	616	492	164	145
Tertile 3	568	222		0.023*	0.021*	904	612	183	801	257
White meat										
Tertile 1	—	—	—	—	—	—	—	—	—	—
Tertile 2	369	348	288	849	678	338	426	566	727	736
Tertile 3	199	334	530	306	421	0.048*	830	817	190	266
Lunch meat										
Tertile 1	—	—	—	—	—	—	—	—	—	—
Tertile 2	1.000	0.024*	803	582	674	250	245	982	503	728
Tertile 3	179	0.004*	773	833	267	453	605	457	420	631

Table 5 (continues). Data obtained from a bivariate analysis between cytogenetic markers and different food groups in peripheral blood lymphocytes (PBL) and buccal mucosa cells (BMC)

	PBL			BMC						
	MNi	**NBUDs**	**NPBs**	**MNi**	**NBUDs**	**BN**	**CC**	**KHC**	**PYK**	**KYL**
Cereals										
Tertile 1	—	—	—	—	—	—	—	—	—	—
Tertile 2	553	216	657	904	354	88	686	619	25	64
Tertile 3	604	845	136	808	167	309	148	780	74	233
Fat and oil										
Tertile 1	—	—	—	—	—	—	—	—	—	—
Tertile 2	0.020*	198	695	311	0.009*	557	64	528	332	743
Tertile 3	677	83	785	613	346	108	250	411	173	939
Candies										
Tertile 1	—	—	—	—	—	—	—	—	—	—
Tertile 2	834	169	771	142	515	487	456	696	885	908
Tertile 3	77	355	685	678	302	325	317	785	540	728

[a] Only two categories of consumption were created for PBL NPBs according to the median

*Significant difference

MNi: micronuclei; NBUDs: nuclear buds; NPBs: nucleoplasmic bridges; BN: binucleated cells; CC: cells with condensed chromatin; KHC: karyorrhectic cells; PYK: pyknotic cells; KYL: karyolytic cells.

the frequency of MNi in exposed minors (Baier *et al.*, 2002). These contradictory results could be explained partly by the influence of genetic polymorphisms that confer protection or susceptibility to genotoxins (Chandirasekar *et al.*, 2011; Fenech and Bonassi, 2011).

Due to the increase in the number of human biomonitoring studies that use the BMCyt assay, it is necessary to know the influence of external variables on the biomarkers measured by the test. A pooled analysis of the results of this method in 5,424 individuals, including around 725 children, concluded that sex does not influence the values of MNi, NBUDs, BN or PYK cells; however, they found a higher number of KHC cells in males (Bonassi *et al.*, 2011a). It should be noted that some studies in this review reported only the frequency of MNi as a biomarker. Additionally, it was mentioned that the values of cells with CC and KYL were so heterogeneous that they were not taken into account in the statistical analysis. These same authors revealed a positive association between age and the frequency of MNi, KHC and PYK. In contrast, in our study only one relationship was observed with NBUDs; however, the statistical significance of this association is so close to the threshold of the set level of significance ($p<0.05$) that it may wear off if the sample size is increased. Furthermore, it appears that changes in baseline levels of markers in epithelial cells, or at least of MNi, are only evident among individuals with a wide age difference (Hopf *et al.*, 2020).

In concordance with our study, no evidence on the influence of weight status on biomarkers in BMC was observed in children by Torres-Bugarin *et al.* (2009); however, the frequencies of the measured parameters were notably different from those obtained in our study. A similar result was reported by Espinosa Arreola *et al.* (2019), who included the nuclear anomalies - except MNi - in a single variable. Additionally, Villarini *et al.* (2018) showed no connection between body mass index (BMI) and this biomarker in oral epithelial cells of minors. On the contrary, Idolo *et al.* (2018) found that obesity had a positive influence on the occurrence of MNi in BMC from a group of children. These results indicate that although obesity is related to an increase in reactive oxygen species (ROS) which induce DNA damage (Usman and Volpi, 2018), it is not clear whether this can be evidenced by cytome assay markers, since most studies about this topic do not take into account the influence of potential confounding factors from diet (Setayesh *et al.*, 2018).

Regarding the exposure to SHS in children and the outcomes of the method, two studies found a positive association between the value of MNi and having a mother who smokes. However, these reports did not fit a multivariate analysis for the rest of the markers (Idolo *et al.*, 2018; Villarini *et al.*, 2018). Following this, Bonassi *et al.* (2011a) reported higher levels of MNi and PYK in heavy smokers (40 cigarettes per day), which could

Table 6. Data obtained from a multivariate model between the genetic damage markers in peripheral blood lymphocytes (PBL) and the consumption level of different food groups.

	MNi			NBUDs			NPBs		
	Percentage variation	95% CI	*p*	Percentage variation	95% CI	*p*	Percentage variation	95% CI	*p*
Tropical fruits									
Tertile 1	N/A	N/A	N/A	N/A	N/A	N/A	0	—	—
Tertile 2							-72.00	0.04/1.23	87
Tertile 3							-42.00	0.05/1.15	75
Fruit vegetables									
Tertile 1	N/A	N/A	N/A	0	—	—	N/A	N/A	N/A
Tertile 2				7.50	0.31/3.35	986			
Tertile 3				112.50	0.60/5.19	297			
Leaf vegetables									
Tertile 1	N/A	N/A	N/A	0	—	—	N/A	N/A	N/A
Tertile 2				-78.72	0.09/2.14	306			
Tertile 3				-59.57	0.21/2.04	466			
Roots and Tubers									
Tertile 1	0	—	—	N/A	N/A	N/A	0	—	—
Tertile 2	-32.39	0.39/0.96	0.032*				-69.57	0.03/1.31	94
Tertile 3	-3.78	0.44/1.17	180				-28.26	0.06/1.34	114
Legumes									
Tertile 1	0	—	—	N/A	N/A	N/A	0	—	—
Tertile 2	-29.61	0.45/1.20	215				233.33		381
Tertile 3	-7.77	0.56/1.47	684				291.67		76
Dairy products and eggs									
Tertile 1	0	—	—	N/A	N/A	N/A	N/A	N/A	N/A
Tertile 2	19.00	0.65/1.77	786						
Tertile 3	46.00	0.67/1.93	625						
Red meata									
Tertile 1	0	—	—	N/A	N/A	N/A	N/A	N/A	N/A
Tertile 2	-48.59	0.38/1.31	273						
Tertile 3	12.60	0.82/1.96	279						
White meat									
Tertile 1	0	—	—	N/A	N/A	N/A	N/A	N/A	N/A
Tertile 2	-26.25	0.42/1.68	616						
Tertile 3	30.97	0.82/1.85	323						

Table 6 (continues). Data obtained from a multivariate model between the genetic damage markers in peripheral blood lymphocytes (PBL) and the consumption level of different food groups.

	MNi			NBUDs			NPBs		
	Percentage variation	95% CI	*p*	Percentage variation	95% CI	*p*	Percentage variation	95% CI	*p*
Lunch meat									
Tertile 1	0	—	—	0	—	—	N/A	N/A	N/A
Tertile 2	0	0.50/1.31	378	-90.09	0.02/1.31	88			
Tertile 3	-27.01	0.48/1.22	265	-88.29	0.05/1.14	72			
Cereals									
Tertile 1	N/A	N/A	N/A	N/A	N/A	N/A	0	—	—
Tertile 2							53.33	0.12/6.77	933
Tertile 3							233.33	0.40/19.56	297
Fat and oil									
Tertile 1	0	—	—	0	—	—	N/A	N/A	N/A
Tertile 2	-61.26	0.22/1.16	105	-74.36	0.05/3.49	415			
Tertile 3	-8.23	0.65/1.59	934	-62.82	0.15/2.05	383			
Candies									
Tertile 1	0	—	—	0	—	—	N/A	N/A	N/A
Tertile 2	-5.86	0.70/2.32	435	-78.00	0.07/7.15	765			
Tertile 3	49.19	0.99/2.55	56	58.00	0.59/4.65	339			

Percentage variation and *p*-values refer to comparison with the first tertile of consumption.

N/A: Not applicable. The variable did not meet the necessary requirements to be included in the multivariate analysis

* Significant difference

MNi: micronuclei; NBUDs: nuclear buds; NPBs: nucleoplasmic bridges; CI confidence interval.

have an analogy with our results; however, it should be taken into consideration that Argentine adolescents were affected by passive smoking, therefore the type of exposure was different. On the other hand, the value of significance for the relationship with PYK in our case was close to the threshold of the set significance level. Interestingly, Nersesyan *et al.* (2011) found controversial results regarding the relationship between nicotine exposure in smokers and BMCyt assay biomarkers, indicating that, in order to determine the association between these variables, controlled intervention trials on smokers are necessary.

It is known that diet provides the necessary nutrients for DNA synthesis and repair, and some foods are even involved in the prevention of genomic damage. Consequently, the evaluation of dietary habits and food-related factors should be considered in biomonitoring studies that use biomarkers of genotoxicity (Fenech, 2020). However, some researchers do not take these parameters into account or do not include them in the statistical analysis when the focus of their research differs from a dietary topic. Such shortcomings should be addressed since it has already been observed that a high intake of vitamin E, retinol, folate, nicotinic acid and calcium decrease the frequency of MNi in adults, while a high consumption of riboflavin, pantothenic acid and biotin increases this value. Additionally, it is known that both the deficiency and excess of micronutrients can be harmful (Fenech *et al.*, 2005).

It is worth mentioning that in the consulted databases and search engines (PubMed, ScienceDirect and Google Scholar) no reports were found on the influence of the intake of macronutrients or specific food groups on the cytome assay markers in PBL from children. Some studies related to micronutrients were evidenced, for instance, Milne *et al.* (2015) found a positive association

Table 7. Data obtained from the analysis of the association between the consumption level of different food groups and markers of genotoxicity and cytokinesis failure in buccal mucosa cells (BMC)

	MNi			NBUDs			BN		
	Percentage variation	95% CI	*p*	Percentage variation	95% CI	*p*	Percentage variation	95% CI	*p*
Citrus									
Tertile 1	0	—	—	0	—	—	0	—	—
Tertile 2	-48.45	0.33/2.70	915	157.14	0.81/4.02	151	111.67	0.88/4.11	100
Tertile 3	-55.88	0.37/2.82	960	122.14	1.14/5.70	0.022*	-16.67	0.23/1.52	276
Tropical fruits									
Tertile 1	0	—	—	N/A	N/A	N/A	N/A	N/A	N/A
Tertile 2	-13.03	0.33/2.48	845						
Tertile 3	-83.35	0.08/0.67	0.007*						
Fruit vegetables									
Tertile 1	N/A	N/A	N/A	0	—	—	N/A	N/A	N/A
Tertile 2				131.82	0.80/4.79	139			
Tertile 3				169.70	0.65/4.84	267			
Leaf vegetables									
Tertile 1	0	—	—	N/A	N/A	N/A	N/A	N/A	N/A
Tertile 2	-69.84	0.19/1.66	295						
Tertile 3	-30.59	0.31/2.33	761						
Legumes									
Tertile 1	0	—	—	N/A	N/A	N/A	N/A	N/A	N/A
Tertile 2	132.94	1.44/12.61	0.009*						
Tertile 3	72.51	0.66/4.78	255						
Red meat									
Tertile 1	0	—	—	0	—	—	0	—	—
Tertile 2	-9.38	0.13/1.33	139	-7.89	0.51/3.65	530	-77.78	0.06/1.30	105
Tertile 3	-68.98	0.10/0.68	0.006*	175.00	1.42/7.96	0.006*	-4.44	0.45/1.78	752
White meat									
Tertile 1	N/A	N/A	N/A	N/A	N/A	N/A	0	—	—
Tertile 2							80.43	0.78/7.31	126
Tertile 3							126.09	1.46/6.98	0.004*
Cereals									
Tertile 1	N/A	N/A	N/A	0	—	—	0	—	—
Tertile 2				-38.61	0.13/0.72	0.007*	-70.00	0.20/1.56	265
Tertile 3				-48.89	0.08/0.47	0.0002*	-35.24	0.31/2.01	622
Fat and oil									
Tertile 1	N/A	N/A	N/A	0	—	—	0	—	—
Tertile 2				-94.94	0.01/0.54	0.011*	-30.21	0.45/4.67	538
Tertile 3				-32.74	0.52/2.08	913	-51.04	0.20/1.32	168
Candies									
Tertile 1	0	—	—	N/A	N/A	N/A	N/A	N/A	N/A
Tertile 2	-60.71	0.12/1.55	200						
Tertile 3	24.71	0.45/2.87	782						

Percentage variation and *p*-values refer to comparison with the first tertile of consumption.
N/A: Not applicable. The variable did not meet the necessary requirements to be included in the multivariate analysis.
* Significant difference

MNi: micronuclei; NBUDs: nuclear buds; BN: binucleated cells; CI: confidence interval

Table 8. Data obtained from the analysis of the association between the consumption level of different food groups and the baseline frequency of cell death biomarkers in buccal mucosa cells (BMC)

	CC			KHC			PYK			KYL		
	Percentage variation	95% CI	*p*	Percentage variation	95% CI	*p*	Percentage variation	95% CI	*p*	Percentage variation	95% CI	*p*
Tropical fruits												
Tertile 1	N/A	N/A	N/A	0	—	—	N/A	N/A	N/A	N/A	N/A	N/A
Tertile 2				35.09	0.91/2.09	130						
Tertile 3				6.25	0.75/1.67	576						
Fruit vegetables												
Tertile 1	N/A	N/A	N/A	0	—	—	0	—	—	N/A	N/A	N/A
Tertile 2				40.72	0.74/1.76	539	212.50	0.55/6.49	314			
Tertile 3				-6.58	0.46/1.20	226	90.62	0.48/5.79	427			
Leaf vegetables												
Tertile 1	N/A	N/A	N/A	N/A	N/A	N/A	0	—	—	0	—	—
Tertile 2							-42.00	0.18/2.19	460	15.33	0.76/2.85	249
Tertile 3							100.00	0.74/5.37	173	-35.56	0.43/1.56	548
Roots and Tubers												
Tertile 1	N/A	N/A	N/A	N/A	N/A	N/A	0	—	—	N/A	N/A	N/A
Tertile 2							156.41	0.55/4.81	384			
Tertile 3							28.21	0.22/2.34	585			
Legumes												
Tertile 1	0	—	—	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Tertile 2	-45.82	0.40/1.05	80									
Tertile 3	06.02	0.77/1.87	413									
Red meat												
Tertile 1	N/A	N/A	N/A	0	—	—	0	—	—	0	—	—
Tertile 2				18.46	0.65/1.67	852	129.17	0.73/5.50	178	70.75	0.88/3.67	111
Tertile 3				26.69	0.95/2.28	84	14.58	0.30/2.37	750	39.43	0.79/2.52	244
White meat												
Tertile 1	N/A	N/A	N/A	N/A	N/A	N/A	0	—	—	N/A	N/A	N/A
Tertile 2							-28.26	0.07/2.41	331			
Tertile 3							89.13	0.59/3.49	430			
Cereals												
Tertile 1	0	—	—	N/A	N/A	N/A	0	—	—	0	—	—
Tertile 2	-9.05	0.58/1.51	778				300.00	0.44/6.27	448	-45.93	0.25/1.01	53
Tertile 3	-26.86	0.45/1.13	151				196.00	0.49/7.08	356	-30.36	0.42/1.58	544
Fat and oil												
Tertile 1	0	—	—	N/A	N/A	N/A	0	—	—	N/A	N/A	N/A
Tertile 2	-42.92	0.38/1.20	182				-66.00	0.03/2.49	256			
Tertile 3	-20.36	0.62/1.35	639				90.00	0.51/2.92	651			

Percentage variation and *p*-values refer to comparison with the first tertile of consumption.

N/A: Not applicable. The variable did not meet the necessary requirements to be included in the multivariate analysis.

CC: cells with condensed chromatin; KHC: karyorrhectic cells; PYK: pyknotic cells; KYL: karyolytic cells; CI: confidence interval.

between plasma calcium and MNi, while NPBs showed a positive relationship with lutein and a negative relationship with α-tocopherol. Additionally, Prá *et al.* (2011) observed a negative correlation between iron intake and MNi and NPB values. In our approach, the negative association between a moderate consumption of tubers and roots and the frequency of MNi could be due to the phytoconstituents of these foods, including phenolic compounds, which have known antioxidant activity (Chandrasekara and Josheph Kumar, 2016).

The influence of diet on the BMCyt biomarkers was also evaluated in the pooled analysis by Bonassi *et al.* (2011a), who reported that people who consumed fruits daily had a lower frequency of MNi compared to those who did not. This finding is consistent with our results, i.e., the high intake of tropical fruits had a negative effect on this parameter, possibly due to their antioxidant properties (Lim *et al.*, 2007). On the other hand, research on children from Italy showed that the intake of red or processed meat more than four times a week was positively associated with the occurrence of MNi (Idolo *et al.*, 2018), while adherence to the Mediterranean diet would have a negative effect on this outcome (Panico *et al.*, 2020). In contrast, in the group of Argentine adolescents, a high consumption of red meat had a negative influence on MNi; however, this dietary habit increased the number of NBUDs by up to 175%, which would imply another pathway of expression of DNA damage and corroborate a possible genetic risk caused by excessive intake of this food (Wolk, 2017).

On the other hand, our findings showed a negative association between the intake of cereals and the basal level of NBUDs, which could be associated with the phenolic acids and flavonoids contained in these products (Žilić *et al.*, 2011). It was also observed that the consumption of legumes and citrus fruits had a positive influence on genotoxicity markers. This might seem confusing since both types of foods also contain molecules that act as scavengers for free radicals, preventing their binding with the DNA (Amarowicz and Pegg, 2008; Mahmoud *et al.*, 2019). However, the following three remarks should be considered here. 1) Food contains different types of compounds, not just those that act as antioxidants. Citrus pectin, for instance, is related to an increase in ROS that leads to apoptosis in cancer cells (Salehi *et al.*, 2018). 2) The metabolism of each individual is different due to genetic polymorphisms, which intervenes in the use of nutrients from the diet and its relationship with various diseases (Loktionov, 2003). 3) The amount of macro and micronutrients that enter the body depends on the quality and type of food consumed, for example, lactic fermentation of fruits and vegetables changes the functional properties of these foods (Septembre-Malaterre *et al.*, 2018).

In addition to the genotoxicity biomarkers, BMCyt evaluates other parameters, including the frequency of BN cells. This type of nuclear abnormality could indicate failures in cytokinesis as a result of chromosomal nondisjunction (Shi and King, 2005); however, its biological significance is still unknown, so the association of this marker with the ingestion of white meat is disconcerting. Similarly, there is a lack of information regarding the cell death process associated with CC, KHC, KYL and PYK. These types of cells are found in the superficial layers of the oral epithelium and migrate towards the oral cavity in a process of turnover that culminates in the exfoliation of old cells (Thomas *et al.*, 2009). This tissue composition could be affected by the development of oral dysplasia, a phenomenon associated in part with diet (Morse *et al.*, 2000). Nevertheless, Sánchez-Siles *et al.* (2014) found that while some cell death markers were not altered in patients with oral leukoplakia and dysplasia, the parameters of genotoxicity and cytokinesis failure were significantly increased. This would indicate that biomarkers related to cell death are less prone to the influence of exogenous factors, possibly due to the dynamics of the tissue, which is constantly replaced to protect itself against injuries and maintain its structure (Squier and Kremer, 2001).

CONCLUSIONS

The results of this study provide information on the baseline frequencies of the cytome assay biomarkers in two different cell types from a group of Argentine adolescents. This information is relevant not only to establish the cytogenetic status of the study group, but also because it serves as a starting point for the creation of a database about the health status of children in Argentina, with a focus in populations for which currently little information is available, such as those located in rural areas.

Additionally, the multivariate model helped to assess the association between the biomarkers of both versions of the cytome assay and the main demographic characteristics and dietary habits of the study participants. It was determined that exogenous variables (exposure to SHS and diet) had an influence on markers, particularly those of genotoxicity, both in PBL and BMC. In contrast, the consumption of the evaluated food groups had no effect on parameters related to cell death in epithelial cells. These findings help to better understand the influence of potential confounding factors on the results of the cytome assay and confirm that these variables should be included not only in a descriptive analysis, but also in a multivariate one, especially in child biomonitoring studies.

Finally, it should be noted that the findings of this study are subject to one limitation, that is, the sample

size, which could be seen as small in order to generalize the results to a larger population. However, it is worth mentioning that many of the associations found between the biomarkers and the potential confounding variables were considerably below the set significance value. In other words, it is possible that the size of these effects is large enough to be seen in a small sample. Further research focused on the influence of potential confounders on the cytome assay biomarkers in larger samples of children from different areas is recommended.

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