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Pregnancy, maternal exposure to hair dyes and hair straightening cosmetics, and early age leukemia

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ABSTRACT

Objective: To investigate the association between maternal exposure to hair dyes and hair straightening cosmetics (HDSC) during pregnancy and leukemia at an early age (<2 yr., EAL).
Methods: A multicenter hospital-based case-control study was carried out in 13 states in Brazil between 1999 and 2007. Mothers of 176 ALL (acute lymphocytic leukemia) and 55 AML (acute myeloid leukemia) cases and 419 controls were enrolled and interviewed. Data on maternal exposure to HDSC occurring 3 months before pregnancy, during pregnancy and during breastfeeding were obtained. Data were also gathered on paternal exposure to HDSC before pregnancy. Unconditional logistic regression was performed and odds ratios (OR) on the association between HDSC use and EAL were obtained after adjustment for hormonal intake during pregnancy, maternal age, education, birth weight, and the child skin color.
Results: An adjusted OR of 1.78 (95% C.I. 1.13–2.81) was observed between maternal exposure to HDSC in the first trimester of pregnancy and ALL. Regarding AML, an adjusted OR of 2.43 (95% C.I. 1.13–5.22) was found for maternal exposure to HDSC during breastfeeding. No association between maternal exposure to HDSC during pregnancy and ALL or AML was observed in children with *MLL* (Mixed Lineage Leukemia) gene rearrangement.
Conclusions: Results in this study seem to support the hypothesis that maternal exposure to HDSC during pregnancy may be involved in the etiology of leukemia in children under 2 years of age.

1. Introduction

Leukemia is the most common childhood neoplasm, corresponding to about 35.8% of cancer diagnosed in children worldwide [1]. Its etiology seems to be characterized by multiple risk factors involving the interaction between genetic and environmental factors [2,3].

Exposure to carcinogenic substances during pregnancy can be harmful for the fetus, because during this critical window of development, the hematopoietic system is composed of cells in different maturity stages and receives constant stimuli for proliferation, differentiation, and cellular division [3]. Intrauterine exposure to several chemical substances, such as dypirone, estrogens and other medications [4,5], tobacco smoke [6,7], pesticides [5] and organic solvents [8], have been associated with infant leukemia. Furthermore, studies on infant leukemia may be useful to identify relevant environmental risk factors, considering the short latency period between maternal chemical exposures during pregnancy and the development of the disease [9]. In particular, the effects of maternal exposure to certain chemicals, such as those present in hair dyes and hair straightening cosmetics (HDSC), have not been explored in relation to the development of leukemia in the offspring.

There was increased interest in investigating the association between hair dyes and cancer, after their *in vitro* mutagenicity in bacteria, and carcinogenicity in animal cells were reported in 1979 [10]. However, no such associations could be detected in human adults [11,12].

In addition, genetic factors may play an important role in the development of leukemia [5]. In this sense, several studies revealed the importance of the Mixed Lineage Leukemia (*MLL*) gene in hematopoiesis, gene regulation and development [3,5,9]. In fact, somatic *MLL* gene rearrangement is a biomarker of infant acute lymphoblastic leukemia (ALL) [3,13,14].

Considering the evidence that the fetus would be vulnerable to different chemicals crossing the placental barrier [5,9], this study

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aimed to explore the association between maternal exposure to HDSC during pregnancy and the development of early age leukemia (EAL) in their offspring.

2. Methods

2.1. Study population and design

This study is part of the "Multi-Institutional Study of Infant Leukemia: Contribution of Immunomolecular Markers in Distinguishing Different Etiopathogenic Factors", which focuses on incidence and pathogenesis of childhood leukemia (EAL) in Brazil [14]. EAL cases were randomly selected from Brazilian regions through the Brazilian Collaborative Study Group of Infant Leukemia (BCSGIL) [5]. Briefly, the BCSGIL is a multicentric hospital-based case-control study centralized at the Brazilian National Cancer Institute (Instituto Nacional do Cancer, INCA) in Rio de Janeiro, with a network of academic medical centers and hospitals located in 13 different states in Brazil. The BCSGIL has been exploring the association between environmental exposures and the risk of acute leukemia in children younger than 2 years of age. The rationale for including children older than 12 month-old was based on the biological evidence that fusion of somatic genes associated with acute leukemia starts *in utero* [9] and, also, to prevent missing cases with a delayed diagnosis and/or, AML cases with *MLL*-r older than 12 months.

2.2. Inclusion criteria

Patients with leukemia were classified as ALL or AML according to morphology, standard immunophenotyping and molecular biology techniques; *MLL* gene rearrangement was characterized by a cytogenetic-molecular test following a methodology previously described [14].

Controls were selected among children in the same age range, which were hospitalized in the same hospitals or in general hospitals located in the same municipality where cases came from, and undergoing medical treatment for other non-malignant diseases [13,14]. Controls were recruited among children with life-threatening conditions such as infectious and parasitic diseases (33.6%), hematological diseases (13.4%), respiratory diseases (11.7%), congenital anomalies (11.2%), diseases of the digestive tract (10.5%), cardiovascular diseases (6.7%), and other hospitalization causes (12.9%). It is acknowledged in the literature that children relatives with life threatening conditions use to recall illness antecedents in more detail than those of healthy children, thus restraining the introduction of recall bias. Participation of invited cases and controls in the study was, respectively, 96% and 95% [5].

2.3. Exclusion criteria

Cases and controls exclusion criteria were: (1) if children had adoptive parents, unknown or untraced biological parents; (2) children with diagnosis of myelodysplastic syndrome or any congenital syndromes; (3) patients with inconclusive diagnosis of any malignant disease; and finally, patients diagnosed with other malignant tumors were also excluded of both case and control groups.

2.4. Data collection

Information about environmental exposures potentially associated with the leukemogenesis process was collected through a specifically designed and standardized survey. Face-to-face interviews were conducted with the mothers of the cases and controls in order to obtain information on the socioeconomic background of the family, the parents' occupational history, and the lifestyle and family health history. Thus, information was collected on parental exposure to different chemical agents from the preconception period to lactation, including HDSC use.

Self-reported skin color was stratified as white and non-white. Maternal education was stratified as low (8 schooling years or lower), or high (>8 schooling years). Maternal age at birth was stratified according to the literature, and participants recruitment in each region was performed according to local amount of EAL diagnosis.

The variables examined in the present study are those related to maternal HDSC use during the following periods: (i) the three-month interval before pregnancy (preconception period), (ii) pregnancy (1st, 2nd, and 3rd trimesters), and (iii) the three-month period after birth (breastfeeding). HDSC use in each period was categorized as "never", "occasional" (whether the mother reported <2 times), "regular" (2 times or more) and ever (indicating any HDSC exposure) in the aforementioned periods. Paternal hair dyes use refers to any lifetime exposure reported. Whenever HDSC use was mentioned, the commercial brand names were obtained, the respective chemical components further searched and identified. These substances were classified according to their chemical structure (www.pubchem.com) as acids, alcohols, aldehydes, amines, ketones, chlorides, esters, phenols, inorganic substances, and other compounds. Whenever a compound presented multiple functional groups, it was classified according to the predominant one. Then, the effects of the exposure to these chemicals were analyzed using the specific commercialized brands. The commercial names of hair cosmetics regularly commercialized were replaced by alphabet letters ("A", "B", "C") whenever risk estimates on the association between their consumption during pregnancy and EAL in the offspring were presented.

2.5. Statistical analysis

The distribution of frequencies of the studied variables among cases and controls was ascertained using the qui-square test and the Fisher's exact test (two-sided).

After analysis of the studied variables distribution, crude odds ratios exploring their association with EAL were obtained. The variables showing an association with a p-value $\leqslant 0.25$ were selected for further multivariate modeling with unconditional logistic regression. Then, the variables either showing a p-value $\leqslant 0.25$, or those presenting at least a ten percent change on the odds ratio magnitude following their exclusion from the model, were considered as possible confounders and thus retained in the model. Finally, odds ratios (OR) and their respective 95% confidence intervals were obtained through non-conditional logistic regression models (Statistical Package for Social Science, SPSS 15.1 software) after adjustment for selected confounders such as maternal age and education, child skin color, birth weight, oral contraceptives use and pesticides exposure during pregnancy [2,5].

Odds ratios exploring the association between HDSC exposure and EAL were also obtained comparing cases with *MLL* gene rearrangement and controls, adjusted by the aforementioned variables. Socioeconomic level/income [15], explored in our study towards the use of the surrogate variable maternal education, maternal age [16], and race/ethnicity [17], have been reported in the literature as possible risk factors to childhood leukemia.

2.6. Ethical Aspects

This investigation was approved by the Ethics Research Committee of the National Cancer Institute (CEP-INCA, 005/06) and the Ethics Research Committee of the National School of Public

Health, Oswaldo Cruz Foundation (CEP-ENSP, 031/2010). An informed signed consent was obtained from mothers of all enrolled participants (cases and controls).

3. Results

The demographic characteristics of the study participants is shown at Table 1. All participants' enrolment started in January 1999 and ended in December 2007. There were 650 individuals, being 231 cases, of which 176 (76.2%) ALL, and 55 (23.8%) AML, and 419 controls. There were no differences in the geographical distribution of participants, although a higher proportion of white children was observed among cases (64.5%) than controls (36.7%), $p = 0.001$. Mothers of cases showed more schooling years than mothers of controls ($p = 0.001$). Maternal HDSC use during pregnancy was reported by 374 mothers (57.5%) with similar distribution among cases (58.4%) and controls (57.0%). Regarding paternal HSDC use before participants' birth, a positive report was obtained from fathers of 16 (6.9%) cases and 22 controls (5.3%). A total of 126 chemical substances could be traced in the HDSC reported by all participants (Pannel 1).

The analysis of selected commercial brands reported at the interviews revealed the following risk estimates: the commercial brand "A", including 2-amino-3-hydroxypyridine, carbomer, cetrimonium chloride, 2-methyl-5-hydroxyethylaminophenol, ethanolamine, buthylparaben/ethylparaben, m-aminophenol, oleth-30 and polyquaternium-22, showed an adjusted OR = 2.76, 95% C.I. 0.93–8.20 for ALL (10 cases); the commercial brand "B", including citronellol, linalool, propylene glycol, 2-oleamido-1.3-octadecanediol, trideceth-6, methylpropional, oleth-30, hydoxyisoheyl 3-cyclohexene carboxaldehyde, laureth-3, 2-methyl-5-hydroxyethylaminophenol, ethanolamine, 2-amino-3-hydroxypyridine, cetrimonium chloride, chlorhexidine dihydrochloride, hexadimethrine chloride, m-aminophenol, p-aminophenol, butylphenyl, sodium stannate, carbomer, alpha-isomethyl ionone, hydroxypropyltrimonium, polyquaternium-22 and tetrasodium pyrophosphate, showed a high risk estimate for AML, with an adjusted OR = 12.5, 95% C.I. 1.44–108.4 (4 cases); the commercial brand "C", including cetrimonium chloride, cetyl esters, chlorhexidine dihydrochloride, ethanolamine, hexadimethrine chloride, lauric acid, m-aminophenol, oleth-30, pentasodium pentetate, polyquaternium-22, propylene glycol and trideceth-6, showed an adjusted risk estimate for ALL, OR = 4.29, 95% C.I. 0.57–3.21 (4 cases).

The ALL risk estimate associated with any maternal use of HDSC during the first trimester of pregnancy revealed an adjusted OR = 1.78, 95% C.I. 1.13–2.81. An adjusted OR = 2.29, 95% C.I. 1.30–4.04 was also observed for those reporting occasional use of these cosmetics in the same period. For AML cases, an adjusted OR = 2.43, 95% C.I.1.13–5.22 was observed among women reporting HDSC use during breastfeeding, and an adjusted OR = 2.59 (95% C.I. 1.01–6.70) for occasional use in the same period (Table 2). No association was found between HDSC exposure during pregnancy and *MLLr* (Table 3).

Table 1
Sociodemographic variables distribution, leukemia cases and controls, children <2 yr., Brazil, 1999–2007.

	Cases ($n = 231$) n (%)	Controls ($n = 419$) n (%)	p-value
Sex			
Male	124 (53.7)	227 (54.2)	0.903
Female	107 (46.3)	192 (45.8)	
Skin color			
White	149 (64.5)	154 (36.7)	0.001
Non-white	77 (33.3)	251 (59.9)	
Maternal age at birth (yr.)			
<19	13 (5.6)	77 (18.4)	0.001
19–24	80 (34.6)	162 (38.7)	
25–30	66 (28.6)	102 (24.3)	
31–36	52 (22.5)	55 (13.1)	
>36	20 (8.7)	23 (5.5)	
Maternal education (yr.)			
<9	125 (54.1)	286 (68.3)	0.001
9 or more	106 (45.9)	133 (31.7)	
Geographic region			
Northeast	52 (22.5)	103 (24.6)	0.911
Midwest	17 (7.4)	32 (7.6)	
Southeast	134 (58.0)	231 (55.2)	
South	28 (12.1)	53 (12.6)	

4. Discussion

The causal association between the exposure to certain chemicals and cancer has been explored and documented throughout the literature [2,9,18]. The International Agency for Cancer Research (IARC) has classified many chemical compounds, such as benzene and dyes metabolized to benzidine as carcinogens to humans (Group 1) [19]. Since the sixties hair dyes and hair cosmetics have gained growing interest due to their potential role on carcinogenesis. Chemical compounds of hair dyes can be absorbed by inhalation and/or skin contact causing skin and respiratory tract long term injuries, or even cancer [20,21]. These substances may cause accumulation of alterations in genes regulating cell differentiation, proliferation, DNA-repair and apoptosis. These changes may lead to the acquisition of a malignant phenotype, thus resulting in cells immortalization, and in consequence ceasing to perform their normal functions in the organism [19]. The association between hair dyes exposure and cancer is controversial, despite existing some evidence supporting the association with the development of hematological neoplasia [21–25]. From a public health perspective, such exposure deserves special attention considering the extensive use of hair dyes by the adult population worldwide, which could result in a high attributable risk for this exposure.

All studies exploring the association between hair cosmetics use and cancer were performed in adults [21–26]. Studies examining the risk of cancer in the offspring of parents exposed to such substances have not been published. However, some studies have provided evidence on the association between infant leukemia and exposure to other chemicals, such as dipyrone, estrogens, pesticides, and organic solvents [2,5]. These investigations suggested that leukemogenesis may be related to the fact that the developing fetus is more vulnerable to DNA damage during the first stages of pregnancy, thus leading to genetic rearrangements. Results of the present study add some evidence to this hypothesis, with an increase in ALL risk estimates in the offspring of mothers who have been exposed to HDSC during the first trimester of pregnancy (OR = 1.78, 95% C.I. 1.13–2.81).

Commercialized HDSC contain a large variety of chemical compounds [12]. The present investigation explored the effects of exposure to these chemicals which were identified in the different products whose commercial names were provided by participants. As a result, 126 chemicals were identified, allowing us to evaluate the association of chemical mixtures with the risk of EAL. Among them, persulphates, organic solvents acting as endocrine disruptors [19], and paraben esters also having endocrine disruption activity [27], are included. One of these compounds, butylparaben ester, has been associated with hematological cancer, lung adenocarcinoma, and osteosarcoma in mice [28]. Butylparaben ester can also interact with DNA, RNA, and proteins, inhibiting cell division in bacteria such as *Bacillus subtilis* and *Escherichia coli* [28].

The compound 2-methyl-5-hydroxyethylaminophenol present in HDSC has shown *in vitro* mutagenic effects in mammalian cells,

Table 2
Maternal exposure to hair dyes and straightening cosmetics during pregnancy, leukemia cases and controls, children <2 yr., Brazil, 1999–2007.

HDSC (pregnancy)	Controls n (%)	ALL n (%)	ALL Crude OR (95% CI)	ALL Adj. OR* (95% CI)	AML n (%)	AML Crude OR (95% CI)	AML Adj. OR* (95% CI)
Reported							
No	180 (43.0)	108 (61.4)	1.00	1.00	28 (50.9)	1.00	1.00
Yes	239 (57.0)	68 (38.6)	0.84 (0.58–1.20)	0.79 (0.54–1.16)	27 (49.1)	1.38 (0.78–2.42)	1.21 (0.64–2.30)
*Pre-conception***							
Never	195 (46.5)	83 (47.2)	1.00	1.00	30 (54.5)	1.00	1.00
Occasionally (<2)	121 (28.9)	55 (31.3)	1.07 (0.71–1.61)	1.10 (0.71–1.71)	9 (16.4)	0.48 (0.22–1.05)	0.65 (0.27–1.53)
Regularly (2 or more)	103 (24.6)	38 (21.6)	0.87 (0.55–1.36)	0.84 (0.52–1.37)	16 (29.1)	1.01 (0.52–1.94)	1.08 (0.51–2.28)
Ever	224 (53.5)	93 (52.9)	0.97 (0.68–1.39)	0.98 (0.67–1.44)	25 (45.5)	0.72 (0.41–1.28)	0.88 (0.46–1.66)
1st Trimester							
Never	339 (80.9)	129 (73.3)	1.00	1.00	43 (78.2)	1.00	1.00
Occasionally (< 2)	41 (9.8)	30 (17.0)	1.92 (1.15–3.21)	2.29 (1.30–4.04)	5 (9.1)	0.96 (0.36–2.56)	1.32 (0.43–4.09)
Regularly (2 or more)	39 (9.3)	17 (9.7)	1.14 (0.63–2.10)	1.25 (0.65–2.41)	7 (12.7)	1.41 (0.59–3.36)	1.97 (0.73–5.38)
Ever	80 (19.1)	47 (26.7)	1.54 (1.02–2.33)	1.78 (1.13–2.81)	12 (21.8)	1.18 (0.60–2.34)	1.71 (0.77–3.82)
2nd Trimester							
Never	346 (82.6)	138 (78.4)	1.00	1.00	47 (85.5)	1.00	1.00
Occasionally (<2)	35 (8.3)	23 (13.1)	1.65 (0.94–2.89)	1.88 (1.03–3.45)	3 (5.5)	0.63 (0.19–2.13)	0.81 (0.21–3.14)
Regularly (2 or more)	38 (9.1)	15 (8.5)	0.99 (0.53–1.86)	1.08 (0.55–2.14)	5 (9.0)	0.97 (0.36–2.58)	1.15 (0.38–3.48)
Ever	73 (17.4)	38 (21.6)	1.31 (0.84–2.02)	1.48 (0.92–2.40)	8 (14.5)	0.81 (0.37–1.78)	1.04 (0.42–2.56)
3rd Trimester							
Never	348 (83.1)	138 (78.4)	1.00	1.00	46 (83.6)	1.00	1.00
Occasionally (<2)	36 (8.6)	23 (13.1)	1.54 (0.87–2.71)	1.73 (0.94–3.19)	4 (7.3)	0.84 (0.28–2.47)	0.89 (0.26–3.02)
Regularly (2 or more)	35 (8.3)	15 (8.5)	1.15 (0.62–2.15)	1.19 (0.61–2.32)	5 (9.1)	1.08 (0.40–2.89)	1.13 (0.37–3.47)
Ever	71 (16.9)	38 (21.6)	1.35 (0.87–2.10)	1.47 (0.91–2.37)	9 (16.4)	0.96 (0.45–2.05)	1.06 (0.44–2.52)
*Breastfeeding****							
Never	341 (81.4)	137 (77.8)	1.00	1.00	39 (70.9)	1.00	1.00
Occasionally (<2)	38 (9.1)	23 (13.1)	1.52 (0.87–2.64)	1.50 (0.82–2.72)	9 (16.4)	2.07 (0.93–4.60)	2.59 (1.01–6.70)
Regularly (2 or more)	40 (9.5)	16 (9.1)	1.00 (0.54–1.85)	1.15 (0.59–2.25)	7 (12.7)	1.53 (0.64–3.65)	1.99 (0.72–5.51)
Ever	78 (18.6)	39 (22.2)	1.29 (0.84–1.98)	1.38 (0.86–2.22)	16 (29.1)	1.79 (0.96–3.37)	2.43 (1.13–5.22)

HDSC – hair dyes and straightening cosmetics.
* ORs adjusted by birth weight, maternal age at birth and education, oral contraceptives and pesticides exposure during pregnancy, and child's, skin color.
** 3 months before birth; *** 3 months after birth.
**** HDSC use in each period was categorized as "never", "occasional" (whether the mother reported < 2 times), "regular" (2 times or more) and ever (indicating any HDSC exposure).

but neither mutagenic or genotoxic effects in *in vivo* tests, nor carcinogenic effects in mice and rats have been observed [29]. From these results it was concluded that there were no reasons for concern about the carcinogenic potential of this compound when used in hair dyes [29]. In the present study, the impact of use during pregnancy or breastfeeding of HDSC containing 2-methyl-5-hydroxyethylaminophenol and paraben ester was investigated. An increased risk estimate was observed for EAL associated with maternal use of HDSC during pregnancy, mainly with the use in the first trimester of gestation for ALL (OR = 1.78, 95% C.I. 1.13–2.81), and during breastfeeding for AML (OR = 2.43, 95% C.I. 1.13–5.22). These increased risk estimates were particularly associated with occasional HDSC use, being higher than those observed among mothers reporting regular HDSC use. However, a dose–effect pattern according to frequency of use was not observed. Some possible explanations can be hypothesized for these findings. At first, such difference can result from HDSC underreported use during pregnancy, mainly the regular use, since it could be socially perceived as inadequate by the mothers who could relate the use of HDSC with adverse health effects. Mazzei and collaborators (2010) evaluated the risk of DNA damage in individuals exposed to homemade formaldehyde-based capillary lotion and other additives, with the result that these compounds have a mutagenic effect in dividing cells [30]. In the opinion of these and other authors, the genotoxic risks of these homemade formulations should be rigorously evaluated, since these products consumers are directly exposed to genotoxic or carcinogenic chemicals [31,32]. Whether these homemade formulations interact with commercialized HDSC, it could partially explain the observation of differences in risk estimates between occasional and regular maternal, since HDSC domestic use may be underreported. Secondly, other explanation could lie in the fact that leukemogenic effect of exposure to these substances may not be cumulative, but rather triggered after reaching a certain exposure threshold. An additional explanatory hypothesis is the fact that a long-term regular use before pregnancy may have upregulated mechanisms for stress defense, DNA repair and detoxification, which may be an advantage for the fetus, compared to the situation in occasional users. Finally, it is worth to consider that the effects of early-life exposure to chemicals may not be characterized by a monotonic dose–response pattern, as observed in adults [33].

The biological associations found in the present study are sustained by Ames and collaborators who reported that hair dye components are mutagenic for bacteria [10]. A comprehensive understanding of the mechanisms of action of HDSC chemical compounds leading to leukemogenesis, whether occurring, still remains unknown. However, it is acknowledged that the accumulation of mutations at regulatory loci at the different stages of differentiation, proliferation, repair and apoptosis may evolve to carcinogenesis [19]. The analysis of leukemic clones may provide information about the event at which the malignant transformation occurred and the types of molecular mechanisms involved [9].

The chemical substances present in hair dyes have been object of evaluation by IARC/WHO. So far, only few of these substances, such as formaldehyde, have been classified as carcinogenic (Group 1), some others such as propylene oxide and chloroform as possible carcinogens (Group 2 B), and several other compounds have been considered as presenting inadequate evidence to evaluate their carcinogenic effects [8,20]. As a whole, the evidence provided by epidemiological studies on the association between hair dyes exposure and cancer is limited, taking into account either the little increase in risk estimates or the observed methodological inconsistencies [34]. Accordingly, some authors have highlighted that further studies on this field should consider the time window of

Table 3
Maternal exposure to hair dyes and straightening cosmetics during pregnancy, *MLLr* cases and controls, Brazil, 1999–2007.

HDSC (pregnancy)	Controls n (%)	*MLLr n* (%)	*MLLr* Crude OR (95% CI)	*MLLr* Adj. OR (95% CI)
*Pre-conception***				
Children 0–11 months				
Never	120 (46.9)	19 (47.5)	1.00	1.00
Occasionally (<2)	75 (29.3)	12 (30.0)	1.01 (0.46–2.20)	0.97 (0.41–2.29)
Regularly(2 or more)	61 (23.8)	9 (22.5)	0.93 (0.40–2.18)	0.80 (0.32–2.03)
Ever	136 (53.1)	21 (52.5)	0.98 (0.50–1.90)	0.89 (0.43–1.83)
Children 12–23 months				
Never	75 (46.0)	11 (50.0)	1.00	1.00
Occasionally (<2)	46 (28.2)	2 (9.1)	0.30 (0.06–1.40)	0.25 (0.04–1.43)
Regularly(2 or more)	42 (25.8)	9 (40.9)	1.62 (0.64–4.14)	2.21 (0.68–7.12)
Ever	88 (54.0)	11 (50.0)	0.93 (0.39–2.23)	0.98 (0.36–2.67)
1st trimester				
Children 0–11 months				
Never	205 (80.1)	29 (72.5)	1.00	1.00
Occasionally (< 2)	27 (10.5)	8 (20.0)	2.09 (0.87–5.05)	1.96 (0.73–5.26)
Regularly (2 or more)	24 (9.4)	3 (7.5)	0.88 (0.25–3.12)	0.50 (0.11–2.20)
Ever	51 (19.9)	11 (27.5)	1.53 (0.71–3.26)	1.22 (0.52–2.83)
Children 12–23 months				
Never	134 (82.2)	18 (81.9)	1.00	1.00
Occasionally (< 2)	14 (8.6)	1 (4.5)	0.50 (0.06–4.05)	0.65 (0.06–6.80)
Regularly (2 or more)	15 (9.2)	3 (13.6)	1.41 (0.37–5.33)	3.96 (0.72–21.91)
Ever	29 (17.8)	4 (18.1)	0.97 (0.31–3.07)	1.83 (0.47–7.16)
2nd trimester				
Children 0–11 months				
Never	208 (81.3)	33 (82.5)	1.00	1.00
Occasionally (<2)	26 (10.2)	5 (12.5)	1.21 (0.44–3.38)	0.94 (0.28–3.11)
Regularly (2 or more)	22 (8.6)	2 (5.0)	0.57 (0.13–2.55)	0.41 (0.09–2.04)
Ever	48 (18.8)	7 (17.5)	0.92 (0.38–2.20)	0.67 (0.25–1.80)
Children 12–23 months				
Never	138 (84.7)	18 (81.8)	1.00	1.00
Occasionally (< 2)	9 (5.5)	1 (4.5)	0.81 (0.10–6.73)	1.17 (0.12–11.62)
Regularly (2 or more)	16 (9.8)	3 (13.6)	1.36 (0.36–5.11)	3.43 (0.61–19.25)
Ever	25 (15.3)	4 (18.1)	1.16 (0.36–3.71)	2.23 (0.54–9.16)
3rd Trimester				
Children 0–11 months				
Never	209 (81.6)	32 (80.0)	1.00	1.00
Occasionally (< 2)	26 (10.2)	4 (10.0)	1.01 (0.33–3.07)	0.64 (0.18–2.29)
Regularly (2 or more)	21 (8.2)	4 (10.0)	1.24 (0.40–3.86)	0.90 (0.26–3.11)
Ever	47 (18.4)	8 (20.0)	1.11 (0.48–2.57)	0.76 (0.30–1.94)
Children 12–23 months				
Never	139 (85.3)	18 (81.8)	1.00	1.00
Occasionally (< 2)	10 (6.1)	1 (4.5)	0.73 (0.09–6.04)	0.80 (0.07–8.53)
Regularly (2 or more)	14 (8.6)	3 (13.6)	1.56 (0.41–5.96)	4.00 (0.72–22.20)
Ever	24 (14.7)	4 (18.1)	1.22 (0.38–3.90)	2.06 (0.51–8.29)
*Breastfeeding****				
Children 0–11 months				
Never	213 (83.2)	31 (77.5)	1.00	1.00
Occasionally (< 2)	20 (7.8)	5 (12.5)	1.71 (0.60–4.91)	1.29 (0.39–4.35)
Regularly (2 or more)	23 (9.0)	4 (10.0)	1.19 (0.39–3.69)	1.03 (0.29–3.61)
Ever	43 (16.8)	9 (22.5)	1.44 (0.64–3.24)	1.16 (0.46–2.90)
Children 12–23 months				
Never	128 (78.5)	17 (77.3)	1.00	1.00
Occasionally (< 2)	18 (11.0)	2 (9.1)	0.79 (0.17–3.69)	0.76 (0.14–4.16)
Regularly (2 or more)	17 (10.4)	3 (13.6)	1.25 (0.33–4.71)	2.28 (0.39–13.48)
Ever	35 (21.4)	5 (22.7)	1.02 (0.35–2.93)	1.21 (0.34–4.34)

MLLr – leukemia cases with *MLL* gene rearrangement.
HDSC – hair dyes and hair straightening cosmetics.
*ORs adjusted by birth weight, maternal age at birth and education, oral contraceptives and pesticides exposure during pregnancy, and child's, skin color.
HDSC use in each period was categorized as "never", "occasional" (whether the mother reported <2 times), "regular" (2 times or more) and ever (indicating any HDSC exposure).
** 3 months before birth.
*** 3 months after birth.

exposure and the role of genetic polymorphisms metabolizing HDSC chemicals [12].

The present study also explored the association between HDSC use and the occurrence of *MLL-r*, since the latter is known to be a somatic mutation for early ALL [9]. No association was observed between maternal exposure to HDSC and either ALL or AML with *MLL*-r. Thus, the investigated chemical compounds may not act in the pathways of the *MLL* gene, but rather leading to modulation of other genes in hematological cells [35].

The current investigation has some limitations. First, no information was collected concerning the lifetime use of maternal hair dyes, the calendar year of starting use, or whether such use was permanent or temporary. Pearson's correlation coefficients (r) between the time windows of exposure were high during pregnancy, i.e., $r = 0.80$ between first and second trimesters, and $r = 0.67$ between first trimester and breastfeeding. Because maternal HDSC reported use seems to have occurred during the whole pregnancy and afterwards, definition of more specific time windows of exposure may be inaccurate. Thus, despite participant mothers have been requested to inform about HDSC use during the index pregnancy, they may have used them for several years, and potential toxic effects of HDSC lifetime consumption were not evaluated in this investigation. In addition, these chemicals are also common components of other cosmetics and personal hygiene items, which were not evaluated in this study. On the other hand, if a recall bias relative to HDSC past use occurred, it probably was not differential between case and control mothers. As usually occurs in hospital-based case-control studies, the investigation design could also have introduced selection bias. However, since cases and controls were recruited from the same municipalities, if not in the same hospitals, and considering that hospital controls were recruited among patients with several life-threatening pathologies, we believe that the occurrence of such biases may be negligible.

Strengths of this investigation include the analysis of a series of cases of a rare disease such as EAL, and the use of a quite detailed questionnaire on HDSC exposure during pregnancy which includes frequency and time window of use (i.e. preconception, pregnancy trimesters, and breastfeeding period).

To our knowledge, this is so far, the first investigation exploring the association between maternal HDSC use during pregnancy and risk of EAL in the offspring. Furthermore, exposure to specific HDSC compounds was for the first time evaluated addressing chemicals mixtures as they are commercialized. In this sense, this study provides some evidence on the high risk of EAL following maternal use of HDSC containing chemical substances previously reported in the literature as possible mutagenic or carcinogenic in animals, such as 2-methyl-5-hydroxyethylaminophenol and parabens.

As an investigation providing some evidence on the association between selected chemical substances and EAL, these results need to be confirmed by studies in other exposed populations. In addition, in order to prevent the potential harmful effects of HDSC use to the developing fetus, the results obtained in this study should be communicated to the general population, particularly to women of reproductive age and pregnant women.

5. Conclusion

This study found some evidence of association between maternal HDSC use during pregnancy and the development of ALL and AML under 2 years of age. The general population, and particularly women, should be informed on the prudent avoidance of HDSC use during pregnancy and breastfeeding.

Conflict of financial interests

The authors declare no competing financial interests.

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Appendix A

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Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cbi.2013.05.012.

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