

SMOKING IN PREGNANCY – THE RISK FACTOR FOR THE DEVELOPMENT OF LIP AND PALATE CLEFTS WITH FETUS

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Summary. *There are a number of factors which may have harmful effects on the fetal development during pregnancy. Particularly critical is the period between the 4th and 12th week of intrauterine development, during which differentiation and determination of the oronasal structures take place. We investigated the influence of maternal smoking during the first trimester of pregnancy on the appearance of lip and palate clefts. The investigation included children divided into case and control groups, as well as their parents. The study group comprised 96 children with anomalies, born in the period from March 1999 to December 2003, whereas the control group comprised 142 healthy children born in the same time period. Cigarette smoking has proved a powerful risk factor for the emergence of the cleft lip and palate. A high percentage of smoking mothers have been observed in the study group (51%). Compared to controls, smoking was statistically more prevalent in the study group ($p < 0.05$). The risk of the appearance of the cleft lip and palate is abruptly increased in the category of daily smoking of over 20 cigarettes. The risk is more than 7 times higher. The results of this study are very similar to those of numerous scientific studies investigating the presence of positive effects between maternal smoking and the appearance of clefts in terms of response to the number of cigarettes smoked per day. The great negative influence of maternal cigarette smoking during pregnancy on the emergence of the cleft lip and palate confirmed by this scientific research as well, implicates the need for most urgent control of smoking in pregnancy, aimed at prevention of the onset of this severe anomaly.*

Key words: *Cigarette smoking, cleft lip and palate, interdependence, statistical analysis*

Introduction

Neural tube defects and lip and palate clefts are classed among the most frequent congenital malformations in humans. Although clefts have been reported for centuries, their etiology has not been clearly established yet. Now, after so many years, the leading scientists are beginning to wonder if this peculiarity may not be too complex to be completely understood. The established opinion is that the etiology of clefts is multifactorial (gene-environment interactions). Heredity is polygenic and accompanied with the existence of a threshold. The acquirement factors may speed up the threshold transgression in susceptible individuals and one of the most important factors is maternal smoking during pregnancy.

The association between maternal cigarette smoking and an increased risk of having a child with a cleft lip and palate was pointed out to in the early 1970s (1). Since then, there has been continued interest in the study of these problems (2-10). The general conclusion is that a majority of studies point out to the positive association between maternal smoking during pregnancy and the occurrence of clefts in their offspring, but its statistical significance has not always been confirmed. The basic confounding factors were maternal age and nutrition. All scientific studies have mostly shown that

the risk increases with the number of cigarettes smoked (a positive dose-response effect). Out of numerous scientific studies published which have concerned these problems, the following one should be singled out in particular: a meta-analysis of ten scientific studies by Wyszynski and Beaty (1966-1996) is one of the most precise studies until now (10).

Lorente *et al.* (8) encompassed children from several European centers, more precisely, 161 children with clefts and 1,134 children as controls. The analyses in this study were multivariate, combining a larger number of factors which could contribute to the occurrence of clefts, and smoking. Chung *et al.* (2) investigated a large study group (2,207 children with clefts) and controls (4,414 children without any congenital malformations). Their results, too, showed that there was a relationship between the number of cigarettes smoked daily and the percentage of cleft occurrence.

Honein *et al.* (11) also pointed out to the detrimental effects of smoking during pregnancy. They found out that cigarette smoking increased the risk for several congenital malformations, including lip and palate clefts.

Owing to advancements in medicine and molecular biology, the effects of smoking during pregnancy on the developing embryo have been recently studied at both the molecular and gene level. The first scientific research to

investigate the association between maternal smoking and genes of the newborn as a risk factor for the occurrence of clefts has isolated the modified transforming growth factor alpha (TGF-alpha) gene allele and shown that some babies (those who carry the modified TGF-alpha) are genetically more susceptible to cleft formation - if the mother smokes (9). Another study has estimated that the risk for smoking mothers whose babies were either heterozygotic or homozygotic for the modified TGF-alpha was twice as high (3). These studies (3,9) have given evidence contributing to the long-term standpoint that the etiology of clefts is multifactorial.

Botto (12) found that the effects of smoking on the occurrence of clefts are dependent upon candidate genes which may happen to be at phase I (activating) and phase II (detoxifying). The danger of their impact is greater when these genes are in the activating phase, because the toxic effect of tobacco is intensified during this phase. These genes include: cytochrome isoenzymes P450 1A1 (CYP1A1) and theta-l-glutathione-S-transferase (GSTT1).

Van Rooij *et al.* (13) have found out that the combination of smoking and the presence of the null allele of GSTT1 in mothers twofold increases the risk for clefts in their children in comparison to the combination of non-smoking and the presence of the common-type GSTT1.

Some other studies have investigated the mechanism of toxic effects of tobacco on the embryo. Mc Nulty (14) concluded that smokers had lower folate levels, and Walmsley *et al.* (15) have established lower red blood counts.

Aim of Study

To examine the occurrence of the lip and palate clefts as a result of mothers' smoking in pregnancy and to determine the risk degree as related to the number of cigarettes smoked daily.

Patients and Methods

The investigation included children divided into a study and control group, as well as their parents. The study group comprised 96 children born with an anomaly – the cleft lip and palate (nonsyndromic clefts). All these children were born in the period from March 1999 to December 2003.

The control group comprised 142 children who satisfied the selectional criteria (being healthy and of the same age as the children from the study group). This group consisted of children attending three kindergartens in Niš.

The investigation of risk factors involved in the occurrence of the cleft lip and palate was conducted by way of two questionnaires. One of them was intended for the mothers, and the second one for the fathers in both of the groups. The questionnaire consisted of questions related to the first trimester of pregnancy of the to-be-mothers.

Our investigation was conducted in the period from December 2002 to December 2003, at the following clinics: Clinic for Gynaecology in Niš, Military-Medical Academy in Belgrade (Institute of Plastic Surgery), Institute of Maternal and Child Care in Novi Beograd, and Pediatric Clinic in Tirsh Street, Belgrade.

The study and control groups were compared for the purpose of obtaining the most cogent illustration of the changing risk value, in relation to the number of cigarettes smoked daily.

The statistical methods used were χ^2 test (Chi square test), and Odds ratios defined as: OR = (odds of exposure among ill)/(odds of exposure among well).

Results

There were altogether 49 mothers (51.04%) who smoked during pregnancy and had children with some kind of a cleft. In comparison to the number of smokers in controls, 53 (37.32%), there was a statistically significant larger number of smoking mothers in the study group ($p < 0.05$) (Fig. 1).

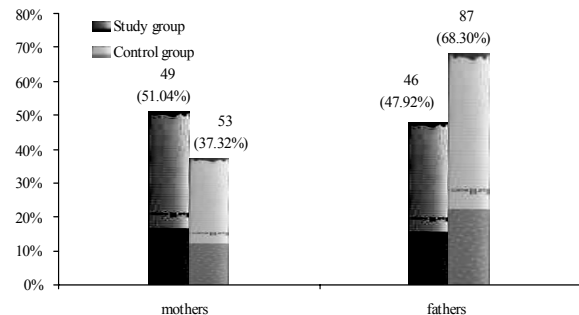


Fig. 1. Survey of frequency of smoking in mothers and fathers in the groups studied, in percentages

In the study group, 47.92% of the fathers were smokers, whereas their percentage is almost 70% in the control group and, in terms of statistics, even significantly higher than the number of nonsmokers ($p < 0.01$).

The largest number of mothers from the study group, 30 (31.25%), who smoked during pregnancy, belonged to the first category (fewer than 10 cigarettes per day), 14 (14.58%) of them belonged to the second one (fewer than 20 cigarettes a day), and the lowest percentage, 5 (5.21%), belonged to the third category (more than 20 cigarettes a day) (Fig. 2).

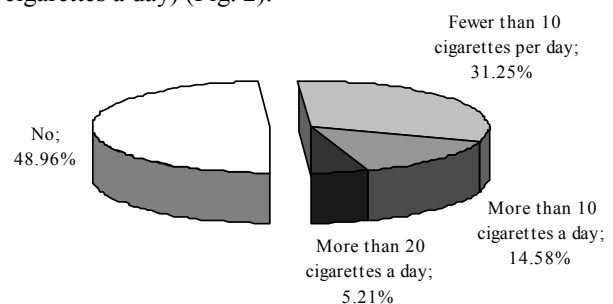


Fig. 2. Frequency of cigarette smoking in the study group

Women who smoke fewer than 10 cigarettes a day are at risk of the occurrence of lip and palate clefts of $OR = 1.39$.

Women who smoke from 10 to 20 cigarettes a day carry the risk of occurrence of lip and palate clefts which is $OR = 3.29$.

Women who smoke more than 20 cigarettes a day are at risk of the occurrence of lip and palate clefts of $OR = 7.75$.

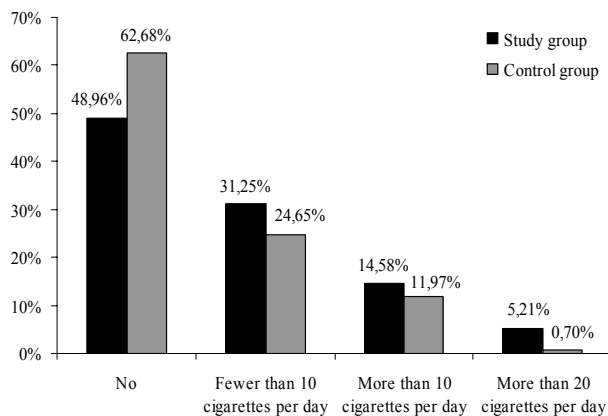


Fig. 3. Survey of frequency of smoking in relation to the number of cigarettes smoked daily, in percentages

Discussion

In the course of our research on the influence of maternal smoking in pregnancy on the occurrence of lip and palate clefts, we obtained some overwhelming data concerning the prevalence of this detrimental habit, even in pregnant women. The fact that smoking is highly prevalent among women in these parts of the world is supported by the data that more than 51% of mothers in the study group and 37.32% of those in controls smoked in the first trimester of pregnancy, a very risky and sensitive period, during which the dynamic processes of organogenesis take place. By comparing the relationship of maternal cigarette smoking between mothers of children with clefts (51.04%) and mothers of healthy children (37.32%), an increased number of smoking mothers in the study group was found to be statistically significant ($p < 0.05$) (Fig. 1). In addition, the odds ratio (OR) is $OR = 1.75$, which means that the mothers who smoked during pregnancy ran a risk for the appearance of lip and palate clefts in their children that was higher by 75%.

Based on these results, it can be concluded that cigarette smoking in the first trimester of pregnancy is a strong risk factor for the occurrence of lip and palate clefts.

The study and control groups were compared for the purpose of obtaining the most convincing illustration of the changing risk value in relation to the number of cigarettes smoked daily (Fig. 3).

Based on the odds ratios (OR) obtained, it can be observed that the risk of the occurrence of lip and palate

clefts increases with the increased number of cigarettes smoked daily. For women who smoke fewer than 10 cigarettes a day the risk is $OR = 1.39$, for women who smoke 10 to 20 cigarettes a day the $OR = 3.29$, and for those who smoke more than 20 cigarettes per day, it is $OR = 7.75$. It is noticeable that this risk is particularly increased, more than 7-fold, in the category of women who smoke more than 20 cigarettes a day.

If we compare the results of our research with those of the research which studied the presence of a positive "dose-response" effect between maternal smoking and the clefts, and in terms of the number of cigarettes smoked daily, it is obvious that the results obtained are very similar.

Wyszynski and Beaty (10) obtained odds ratios of $OR = 1.29$ on the basis of meta-analyses comprising 10 scientific studies carried out in the period from 1966 to 1996, which investigated and confirmed the effects of smoking on the occurrence of lip and palate clefts. This OR, as a mean value of the smoking categories ratios used by previous authors, is lower than the ratios we have obtained, namely, $OR = 1.75$.

Lieff *et al.* (7) have also confirmed the association between maternal cigarette smoking and the occurrence of clefts in the children their studied. In their investigation, they used categories of smoking similar to ours, so these can be compared. For their category "those who smoke little", the obtained OR was $OR = 1.09$, which is close to our category "women who smoke fewer than 10 cigarettes a day", for which we obtained an OR of 1.39. Similarly, for the "moderate smokers" category they obtained an OR of 1.84, while for our category "fewer than 20 cigarettes a day" the obtained ratio is $OR = 3.29$. The third category of "heavy smokers" with the obtained ratio of $OR = 1.85$ cannot be compared to our category of "those who smoke more than 20 cigarettes a day" with its $OR = 7.75$, because of the small number of subjects that our category comprised.

Our results are very similar to those obtained by Lorente *et al.* (8) who, in addition, investigated the joint influence of smoking and alcohol consumption on the occurrence of clefts. They explained the effects of smoking alone as the $OR = 1.79$ value, which is close to the $OR = 1.75$ value obtained in our study.

Sheiner *et al.* (16) estimate that at least 14% of women smoke during pregnancy, which does not correspond to our results, according to which 51% of women in the study group and 37.32% of those in controls, smoked during pregnancy. Shaw *et al.* (9) found that the risk for the occurrence of clefts was twofold in those women who smoked more than 20 cigarettes a day. This result is not in accordance with our result which is estimated for this category of smoking as the risk that is 7 times higher. However, the risk obtained in our investigation should be interpreted with caution, because it was not obtained on a large sample of subjects who smoked more than 20 cigarettes a day.

Our results were compared to the results of Shaw's study (9), which identified the gene responsible for

clefts and, at the same time, opened new fields in research of the etiology of lip and palate clefts. We were not able to make karyograms for the newborn babies in order to determine which type of the TGF- α the babies inherited. Consequently, we can claim that, in our group of subjects, more than 50% of the mothers who smoked during pregnancy did give birth to babies with clefts, but we cannot know whether the clefts appeared owing to existence of the A2-type of this gene in their babies, nor can we estimate the risk for the occurrence of clefts in relation to the presence of the TGF- α A2-type.

Shaw's examination (9) of the influence of "passive" smoking did not lead to any specific results. We investigated the effects of "passive" smoking, i.e. maternal exposure to cigarette smoke, in relation to smoking of their spouses. Our investigations showed that there were slightly more than 47% smoking fathers in the study group, whereas, interestingly, in the control group that number was almost 70% and statistically even higher in significance than the number of non-smokers ($p < 0.01$). The results about "passive" smoking that we obtained are debatable since "passive" smoking was reduced to smoking of the spouse.

Van Rooij *et al.* (13) established that the existence of the null GSTT allele in the mother and presence of smoking led to the occurrence of a cleft (13). We were not able to determine the presence of GSTT1 in our subjects, so our results cannot be compared. Comparisons can be made only in terms of smoking as a risk factor which plays a great role in the occurrence of clefts, and there, our results agree with those of Van Rooij *et al.* (13).

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Our standpoint is that the studies of some authors (3,9,13) give possibilities for some further, more detailed studies which, we hope, will soon identify the main risk factor for the occurrence of this severe anomaly and thereby its prevention.

In their research, Khoury *et al.* (4,5), Walmsley *et al.* (15) and Kato *et al.* (17) have also confirmed the great, negative effect of smoking during pregnancy (which is in agreement with the results of our research), and made a special reference to the lower folate levels in children born to smoking mothers.

In conclusion, it could be said that our study, too, confirmed the great, detrimental effects of smoking, especially smoking during pregnancy. Smoking, as a risk factor, plays an enormous role in the appearance of these severe anomalies, and the data that our country is at the very top on the European scale when cigarette consumption is concerned speaks in favor of the fact that the problem of smoking in the population of all ages in our country is a great one.

Conclusion

The large number of mothers (51%) in our study, who smoked during pregnancy, and in this case, gave birth to children with clefts, is especially disturbing. With this study we, first of all, wish to point out to some consequences of smoking and the risk of smoking during pregnancy. The risk of the occurrence of lip and palate clefts increased with the increased number of cigarettes smoked daily. It is noticeable that this risk is particularly increased, more than 7 times, in the category of women who smoke more than 20 cigarettes a day.

PUŠENJE U TRUDNOĆI - FAKTOR RIZIKA ZA NASTANAK RASCEPA USNE I NEPCA KOD PLODA

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Kratak sadržaj: Postoji veliki broj faktora rizika koji mogu da ostave katastrofalne posledice na razvoj ploda u trudnoći. Posebno je kritičan period između 4. i 12. nedelje intrauterinog razvoja tokom kojeg se odvijaju diferencijacija i determinacija oronazalnih struktura lica. Autor proučava uticaj pušenja majki tokom prvog tromesečja trudnoće na pojavu rascepa usne i nepca. Istraživanje obuhvata decu studijske i kontrolne grupe, kao i njihove roditelje. U studijskoj grupi se nalazi 96-oro dece sa anomalijom, rođene u periodu mart 1999 do decembra 2003. godine. U kontrolnoj grupi se nalazi 142 zdrava deteta, bez anomalije, rođene u istom periodu. Pušenje majki u trudnoći predstavlja snažan faktor rizika u nastanku rascepa usne i nepca. Visok procenat pušenja u trudnoći zabeležen je u studijskoj grupi (51%). U poređenju sa kontrolnom grupom pušenje u trudnoći je bilo statistički značajnije u studijskoj grupi ($p < 0,05$). Rizik od pojave rascepa usne i nepca naglo raste u kategoriji onih žena koje su pušile više od 20 cigareta dnevno. Rizik je više nego 7 puta veći. Rezultati ovog istraživanja su vrlo slični ranijim istraživanjima u ovoj oblasti, koja pokazuju pozitivan efekat između pušenja u trudnoći i pojave rascepa usne i nepca u zavisnosti od broja dnevno popušenih cigareta. Veliki negativni uticaj pušenja u trudnoći na pojavu rascepa usne i nepca koji se pokazao i u ovoj studiji, ukazuje da je potrebno što hitnije suzbijanje pušenja u trudnoći kako bi se sprečila ova teška anomalija.

Ključne reči: Pušenje, rascep usne i nepca, međuzavisnost, statistička analiza