International Journal of Anatomy and Research,
Int J Anat Res 2019, Vol 7(3.2):6851-58. ISSN 2321-4287
DOI: https://dx.doi.org/10.16965/ijar.2019.241

Original Research Article

NON-GENETIC FACTORS CONTRIBUTE TO THE INCIDENCE OF NON SYNDROMIC CLEFTS IN THE CRANIOFACIAL REGION

Betty Anna Jose *[1], Varsha Mokhasi [2], S A Subramani [3], M Shashirekha [4].

*[1] Associate Professor in Anatomy, Vydehi Institute of Medical Sciences and Research centre, Bangalore, Karnataka, India.

[2] Professor in Anatomy, Vydehi Institute of Medical Sciences and Research centre, Bangalore, Karnataka, India.

[3] Professor in Plastic Surgery, Vydehi Institute of Medical Sciences and Research centre, Bangalore, Karnataka, India.

[4] Professor in Anatomy, Vydehi Institute of Medical Sciences and Research centre, Bangalore, Karnataka, India.

ABSTRACT

Background: The multifactorial etiology of clefts includes both genetic and environmental factors. Many studies were conducted to identify the genetic basis of the etiology of clefts and effect of maternal folic acid intake in reducing the risk of clefts.. Not many studies conducted about other environmental factors causing clefts. The present study is to find out the non-genetic factors associated with the nonsyndromic clefts. The maternal periconceptional intake of folic acid, family history, parental age, socioeconomic status, parental alcoholism and smoking, and parental occupational exposure are the factors included in the study.

Materials and methods: The study group comprised 400 subjects with 200 Nonsyndromic cleft cases and 200 healthy controls from the South Indian population. The data was collected in a detailed questionnaire by direct interview and analyzed the data using SPSS version 21. Logistic regression model was used to measure the odds ratio(OR) for the independent variables and Chi- square analysis was performed to find out the significance.

Results: The family history of clefts was found in 10.6% cleft cases (p value= 0.001). The risk of cleft was increased in cases with no maternal folic acid intake in their first trimester of pregnancy (p value= 0.001). Parental age more than 35 years (p value= 0.004) and low maternal education (p value= 0.001) were also found as the risk factors to cleft. Low socioeconomic background was another risk factor (p value= 0.001). Parental occupational exposure in terms of pestcidal exposure was found significant but not the parental medication and smoking.

Conclusion: Maternal consumption of folic acid and multivitamins during the periconceptional period to be assured to prevent the occurrence of oral clefts. Family history of cleft increases the risk of cleft and the risk is further increased when cleft is present in parents or siblings. And maternal age more than 35 years is found more significant than the paternal age . Consanguinity showed 4 fold increase in clefts. Maternal diet is a prime factor as it is directly related to folic acid and vitamin supplementation apart from the socioeconomic status of family.

KEY WORDS: Cleft Lip, Cleft Lip Palate, Cleft Palate, Socioeconomic Status, Non syndromic, Cleft lip/palate.

Address for Correspondence: Dr Betty Anna Jose, Associate Professor in Anatomy, Vydehi Institute of Medical Sciences and Research centre, Bangalore-560066, Karnataka, India. contact no. 9845874881 **E-Mail:** bettyannacj@gmail.com

Access this Article online	Journal Information
Quick Response code	**International Journal of Anatomy and Research**
	ICV for 2016 90.30 — ISSN (E) 2321-4287 | ISSN (P) 2321-8967
	https://www.ijmhr.org/ijar.htm
	DOI-Prefix: https://dx.doi.org/10.16965/ijar
DOI: 10.16965/ijar.2019.241	

Article Information

Received: 01 Jun 2019	Accepted: 08 Jul 2019
Peer Review: 06 Jun 2019	Published (O): 05 Aug 2019
Revised: None	Published (P): 05 Aug 2019

INTRODUCTION

The cleft results from the failure of normal fusion of the tissue during intrauterine life. The clefts in the oral cavity can extend on to the structures on the face or cranium to result in the craniofacial clefts[1]. The most common cleft in the craniofacial region is the cleft Lip and cleft palate. About 70% of oral cleft cases are nonsyndromic and remaining 30% are considered as syndromic clefts. Non-syndromic cleft lip/palate (NSCL/P) is a world wide congenital defect affect about 1 per 700 births [2]. The birth prevalence rate of cleft in India is approximately 27,000 to 33,000 per year [1].

Studies proved that proper maternal diet specially the folic acid intake during the periconceptional period reduces the risk of birth defects. The folic acid has an anti-teratogenic effect and low folic acid intake in animal study showed a delay in secondary palate closure[3]. The non-syndromic cleft has a multifactorial etiology and the involvement of environmental factors especially the maternal environment and the genetic factors either independently or in combination results in clefts were identified[4]. Studies on genetic factors were conducted in India, but not reported the studies on non-genetic factors in non-syndromic clefts.

The present study is to find out the non genetic factors associated with the nonsyndromic clefts in the craniofacial region by comparing the cases with the control group. The maternal periconceptional intake of folic acid, family history, parental age, socioeconomic status(SES), parental alcoholism and smoking, and parental occupational exposure are the factors included in the study.

MATERIALS AND METHODS

This retrospective study included 400 subjects with 200 non syndromic congenital cleft cases of craniofacial region and 200 healthy controls matched for gender, age and locality from the South Indian population. The cleft cases were referred from the Plastic surgery department and the controls were from the Paediatrics and Obstetrics and Gynaecology departments. The patients were clinically examined to rule out the syndromic clefts and was counter checked through their medical records. Approval from the Institutional Ethics committee was obtained. Informed written consent was taken from the study group and from their parents in case of minor. The study group and their parents were interviewed and the data collected in a detailed questionnaire which covers socio-demographic variables, obstetrical history, family history, consanguinity, periconceptional intake of folic acid &vitamin, maternal stress, maternal diet and medication, parental age at time of conception, parental alcoholism, smoking and other teratogenic exposure.

Statistical analysis: The data was analyzed using SPSS version 21. To investigate the role of the factors causing the clefts, a logistic regression model was used to measure the odds ratio(OR) for the independent variables resulting the occurrence of cleft. The odds ratio(OR) is expressed with 95% confidence interval(CI). The level of significance adopted for the analysis was 0.05. The *P* value less than 0.05 was considered statistically significant. Chi- square analysis was also performed to find out the significance.

RESULTS

The clefts in the study group were categorized into 9 craniofacial clefts, 8 facial clefts, 24 cleft lip(CL), 135 cleft lip palate(CLP) and 24 isolated cleft palate(CP) cases. The distribution of cleft was as follows; 4.5% craniofacial clefts, 3.9% facial clefts, 12.3% cleft lip, 67% cleft lip palate and 12.3% cleft palate. In general, 10.6% cleft cases had a family history of clefts (p value= 0.001) and it was not found in control group. The family history of clefts were found in 10.8% of cleft lip palate cases, 22.7% cleft lip cases, 4.5% of cleft palate cases and it was not reported in craniofacial clefts. **(Table 1)**

The low maternal age was more in cases (24.6%) than the control group (8.5%) with p-value =0.001. The paternal age more than 40 years was found in 5.6% cases and it was 0.5% in controls (p value=0.004). Parental age more than 35 years was found more in cleft cases than the control group and was found significant.

According to Kuppuswamy's socioecomic scale[5], 60.9% was poor in cases and it was only 4% in control group(pvalue=0.001). Low parental education was found in 68.15% of cases

Table 1: Shows the significance of nongenetic factors in cases and controls.

Non-genetic factors	present	Cases	Controls	P value
Maternal folic acid intake during pregnancy	No	178(89.4%)	20(10%)	0.001
	Yes	22(10.6%)	180(90%)	
Maternal age	> 35	9(4.5%)	0	
Paternal age	> 35	22(11.2%)	19(9.5%)	0.004
Low maternal education		118(59.2%)	23(11.5%)	0.001
Socioeconomic status	Below average	122(60.9%)	8(4%)	0.001
	Average & above	78(39.1%)	192(96%)	
Family history of cleft	No	178(89.4%)	200(100%)	
	Yes	22(10.6%)	0	

Table 2: Shows association between risk factors and the clefts.

Variable	OR	CI 95%	*P* value
Parental pesticidal exposure	0.523	0.146-1.872	0.319
Socioeconomic status			
Poor	2.609	0.561-12.147	0.001
Average	0.318	0.105-0.967	0.222
Above average			0.043
Maternal sge	0.96	0.880-1.048	0.364
Maternal education	0.715	0.154-3.312	0.668
Low			
Average/medium	0.635	0.187-2.161	0.467
Consanguinity	4.571	1.389-15.040	0.012
Location of residence	6.967	2.705-17.944	0.001

and 16% of control group (pvalue=0.001). It was significant that 74% of cases were from the rural areas, whereas it was 9% in controls**. (Table 2)**

DISCUSSION

The purpose of the study is to compare the environmental or non-genetic factors in cleft cases and controls to assess the risk of occurrence of clefts in South Indian population. In India, there is only limited genetic studies conducted about the clefts and it did not deal with the environmental factors causing clefts. The other studies conducted world wide described only the mutations or its association with maternal folate intake, but not other environmental factors.

Maternal folic acid intake: There are many observational and interventional studies supporting the protective role of folic acid supplementation and prevention of orofacial clefts. Consumption of high doses of folic acid, 6mg or above in the first month of pregnancy showed 50% reduced risk in CP [6] and the intake of the periconceptional folic acid along with multivitamin usage showed more than 50% decrease in cleft lip/palate (CL/P)[7]. When folic acid and multivitamin supplementation used with high folate supplements 64% decrease of risk in CL/P was observed[8]. These studies showed significant protection against recurrence of clefts when mothers were provided with multivitamin supplementation and high dose of folic acid during their periconceptional period. A meta analysis of 37 studies found out that maternal folate and multivitamin supplementation was significantly associated with decreased risk of clefts[9]. In our study highly significant association was found between the risk of clefts and low maternal intake of folic acid during the periconceptional period. About 9%-14% mothers among CL, CLP, CP cases were taken folic acid and there was no folic acid intake among the mothers of cranial and facial cleft cases. But we could not find out significant association between any specific cleft with maternal non intake of folic acid.

The folic acid deficiency acts in various ways that might result in clefts. In folate cycle, 5-Methyl-THF donates methyl groups for the remethylation of homocysteine to methionine. Methionine is required for DNA synthesis and cell growth. Any variation in this DNA synthesis may reduce the cell proliferation rate[10]. The reduced cell proliferation may cause the deficiency and it may result cleft. Hypomethylation can change the transcription and suppression of genes involved in formation of the lip, alveolus, and/or palate. Aberrant DNA methylation during embryogenesis may lead to cleft lip and palate[11].

Decreased synthesis of 5- methyl-THF prevents the remethylation of homocysteine, leads to increased homocysteine level, which in turn is due to low folate intake. Mouse model study showed that homocysteine enters the foetus through amniotic fluid and induces apoptosis in palatal mesenchyme to prevent the fusion of palatal shelves. Thus elevated foetal homocysteine is an important risk factor in causing clefts[12]. We could not correlate our study with the serum homocysteine level as it was a retrospective study. The serum folate and homocysteine concentrations were significantly related. The

low dietary folate intake during the first trimester can cause damaging effect on the organogenesis [10]. Decreased folate intake and maternal hyperhomocysteinemia are related to improper methylation and inadequate DNA synthesis in mother may harm the embryo could be the risk factors for clefts. So sufficient folic acid supplementation can prevent the teratogenic effect of hyperhomocysteinemia.

A population based cohort study identified 4 fold risk for cleft lip for infants of mothers who did not take folic acid during the first 3 months of pregnancy, when compared with those who had a folate intake during the first trimester. The non intake of folic acid during the first 3 months of pregnancy was significantly associated with the occurrence of cleft lip and palate[13]. The folate intake during the first 3 months is a very essential requirement, as the fusion of lip completes by 48th day and palate by 60th day of embryonic life[14]. Present study showed a 5-fold increased risk of clefts for the children of mothers who did not use folic acid in their periconceptional period.

Any perturbation in the folate metabolism pathway can result in folate defeciency and it affects the nucleotide synthesis, cell division, and tissue growth and hampers the craniofacial development. The high levels of homocysteine may also affect developmental activities such as neural crest cell motility and migration, which are important in early development[15]. A study in Mexico showed maternal malnutrition like low vitamin B12 level during pregnancy was a predisposing factor to the incidence of clefts[16]. Maternal diet plays an important role in the folate supplementation. If there is no separate folic acid intake, it can be compensated by the diet rich in folate. All these studies showed that the usage of folate rich diets and folic acid supplements and multivitamins or folic acid supplementation of 400 µg/day or above during early pregnancy reduced the risk of clefts.

The present study showed that the mothers of 91% CL cases, 88% CLP and 86% CP did not take folic acid in first trimester of pregnancy. No mothers of craniofacial and facial cleft cases used folate whereas 89.4% mothers in the control group have taken up folate and it was highly significant. Our study also found that folic acid non intake is significantly associated with the risk of clefts.

Parental pesticidal exposure: The parental agricultural work causes exposure to the teratogenic organic solvents and the agricultural chemicals. Pesticidal exposure to parents is a risk factor causing the clefts in the craniofacial region especially the CLP [16]. In the present study the pesticidal exposure of parents were observed in 55.3% cases and in controls it was only in 4.5%. This parental pesticidal exposure was significantly associated in occurrence of clefts.(p value<0.001). In our study the odds ratio showed slight increase in risk (OR= 0.523, for 95% confidence interval (0.146 to 1.872). The parental pesticidal exposure was found more in CLP (55.8%) than the other clefts (p value=0.041)[17].

The indirect pesticide exposure can happen due to residential proximity and frequent entry to the field after spraying the pesticides. The pesticide residues are present around the area of application especially on soil for some time after spraying. There will be an additional effect of direct exposure when spending more time in pesticide exposed area soon after the pesticide spray. This exposure to the harmful pesticides should be avoided by the humans during the reproductive age especially mother in her first trimester of pregnancy as it is a period of organogenesis and is vulnerable to teratogens. The first trimester is the period of organogenesis and the face formation is occurring during the initial 60 days of embryonic period [14] and teratogenic exposure during this period may result in cleft.

Consanguinity: A significant association was found between consanguinity and clefts, especially with second degree consanguinity. First degree consanguinity was not observed in study group. The first degree consanguinity is the relationship between parent-child or brother-sister. The risk of abnormality is 3-5% in third degree consanguinity, whereas it is increased to 5-10% in second degree consanguinity. No specific cleft group showed association with consanguinity. The abnormality is reducing as the degree of consanguinity increases. This is due to the reduction in the sharing of common genes inherited from the ancestor[18].

In our study consanguinity was identified in 36.9% cases whereas it was observed only in 6% among controls. Consanguinity was found as a significant in causing clefts. It was found a four fold increased risk in clefts in consanguineous marriage. Odds ratio 4.571, 95%CI , 1.389 to 15.040[19].

Family history: Nouri et al opines that family history with a cleft is a significant genetic predisposing factor in the aetiology of cleft lip and palate[20]. There was significant association observed in the occurrence of CL /P than the CP if there is a positive family history with clefts [21]. Our study showed 10.6% of cleft patients had a family history of clefts and it was found more in CLP(7.2%) followed by CL (2.7%) and CP (0.5%). Identical result was found in another study from Gujarat where 14.4% cleft cases had a family history of oral clefts and these positive family history cleft cases was found more in the CLP followed by CP [22]. Our study included CL cases apart from the CLP cases. The risk of occurence of cleft is increased to 3-7% when parent is affected and risk is increased to 15-16% when one parent and sibling are affected[22]. This indicates that the risk of occurrence of cleft is increased when there is more familial cases of clefts. In the present study there was no family history of cleft in control group. No family history of cleft found in the cranial and facial cleft cases.

Birth order: In the present study, the birth order was one in 44.5%, two in 42.5%, three in 7.8%, four in 2.8%, five in 1.7% and 6 in 0.6%. Our study result showed that as the parity increases the risk of cleft is decreasing. The more number of clefts belonged to the birth order 1 or 2. This does not mean that as the birth order is decreasing the chance of cleft is increasing. The fact is that majority of the family has one or two children. Because of this reason, this study group is not sufficient to show the association of cleft and birth order. To justify this conclusion, the study group should include family with 5 or more than 5children.

Many studies identified that the clefting is significantly associated with abortion rate and parity[18,23]. It also showed that among the cases the birth order was higher than the controls[23]. The risk of clefts increases with parity might be due to deficiency of micronutrients and other nutritional supplements as the parity increases. This lights on to that the risk of cleft increases as the birth rank increases. But our study group was inadequate to associate the risk and increased parity.

Parental age: In a previous study, mothers with an age of 39 years or more showed two fold increased risk to have a child with cleft when compared with mothers with 25-29 years old. Paternal age above 40 years and maternal age above 35 years were associated with the risk of occurrence of orofacial clefts[24]. In an Iranian study, it was found that among 36% of cleft cases, the maternal age was between 31-37years during pregnancy[25]. This sheds light on that no risk of cleft when maternal age is 30 or below. In our study, majority of the parents were under the age group of 20-35 years. It was found that in case group 87% fathers and 90.5% in control group were in the age limit of 20-35years. Paternal age of above 35 years was 11% in cases and 9.5% in control group. No father with less than 20 years found in control group but it was 2% among cases. Thus control group is more with an age limit of 20-35 years which is considered as less risk age. The parents with an age of above 35 years or below 20 years were more in case group than the control group and it was significant. The Mexican study showed that the clefting is significantly associated with parental age and the risk increases from the age of 30 years to 50 years[16].

In the present study, among cases 71% mothers belonged to age group of 20-35 years while in control group it was 91.5%. In control group the remaining 8.5% were less than 20 years old. But in case group 29% were either less than 20 years or more than 35 years which was statistically significant($p = 0.001$). Similar result was found in a Chinese study, in which the risk of cleft significantly increased when maternal age was less than 20 years[26].

Parental illness and medication: The environmental factors that are associated with the causation of cleft include vitamin deficiencies particularly vitamins A and B, excess cortisone and ingestion of steroids, anticonvulsants (phenytoin). Excess ACTH (adrenocorticotropic

hormone) in pregnancy especially in the first trimester can result clefts[24]. In our study the maternal drug intake was not found in control group but there was 5% of mothers of cases had used the drugs during the periconceptional period for hyperthyroidism, hypothyroidism, epilepsy and for allergy. The one case of epilepsy had used phenobarbitol and it was a case of familial cleft. The maternal use of antiepileptic drugs like phenytoin and phenobarbital might affect the folate metabolism which in turn caused increased risk of orofacial clefts[27]. In our study, 8 cases were under medication for hyperthyroidism or hypothyroidism. Among the 5% cases of drug intake, 4.5% mothers had a child with CLP and the remaining one had cleft palate. This result could not find any significant association between cleft and maternal medication. Only six mothers revealed that they were under stress because of some severe family issues. This stress might have increased the corticisteroid level. The maternal stress during pregnancy is directly proportional to the risk of clefts[22], as it increases the production of corticosteroids. It has also been reported that hyperthermia[28] more than 40°C, during the first 8 weeks of gestation had a high-risk of causing facial clefts in the developing fetus. In our study, among the mothers of cleft group, only 1% had fever during the pregnancy, but not sure whether it was in the first trimester.

Parental smoking, alcoholism and occupational exposure: Parental occupational exposure[29], maternal smoking and heavy alcohol intake during pregnancy[30] causes increased risk for orofacial clefts. Maternal tobacco smoke exposure showed slight increase in occurrence of clefts. According to our study, smoking and alcoholism did not show any significant risk role in the causation of cleft. The history of smoking and alcoholism was seen only in 4% of fathers. None of the mothers had the habit of the smoking or using alcohol. The exposure to organic solvents was found in 3% parents of cleft cases, as they were painters and working in fabric dyeing.

Previous studies showed that 64% to 68% cleft cases were from the rural areas than the urban area[25,31]. Most of the parents from rural area were farmers. Their socioeconomic status, pesticidal exposure, parental education and the non intake of maternal folic acid supplementation showed significant association with clefts[25]. In the present study 73.7% of cleft cases were from the rural area and most of the parents were farmers while in control group it was only 9% from the rural area. This shows that socioeconomic status, residential locality, occupational exposure, and pesticidal exposure are interlinked.

Socioeconomic status(SES): Low SES was found as a risk factor causing clefts. The SES affects the maternal diet and life style of parents. The socioeconomic status of a family determines the nutritional status of the children of the family that in turn determines their general health. Lower education, lower occupation, or less income leads to low SES. Greater the socioeconomic deprivation more the susceptibility of orofacial clefts. The incidence of cleft decreases as the SES increases. Several studies supported that there is considerable association between low SES and increased risk of clefts[24,32]. The same result was observed in our study also. In our study among cases SES was low in 61%, average in 28% and above average was observed only in 11% cases. In control group the SES distribution was 4%, 70.5%, 25.5% for poor, average and rich strata. The low SES was more in cases but it was only 4% in control group which indicates the low SES is a risk factor for the cleft ($p = 0.001$). The maternal nutrition is related to the socioeconomic status. The mothers from rural areas are with low SES, poor nutrition, non-intake or reduced folic acid supplementation during the periconceptional period might be causing the cleft. In our study it was more than 70%. The cleft cases from the rural population were socioeconomically weaker than their urban counterparts.

Parental education: In a population based survey to analyze the risk factors and prevalence of birth defects, showed the association of low level education of mothers with increased risk of birth defects including clefts[26]. Our study showed that the maternal education was very low or not educated in 59% of cleft cases whereas in control group low educated mothers were only 11.5%. The parental education was

high in control group. In control group 88.5% of mothers and 79.5% of fathers were educated to higher secondary or above. The low educated fathers in control group were 20% but it was vey high (77%) among cases and it was statistically significanct(p = 0.001). This implies the importance of education which can affect the next generation.The educated parents are very much aware of the requirements of nutrition in intrauterine life, or better life style compensating the nutritional requirement which was absent in poor and uneducated parents.

CONCLUSION

It emphasises the fact that maternal consumption of folic acid and multivitamins during the periconceptional period to be assured to prevent the occurrence of oral clefts. Family history of cleft increases the risk of cleft and the risk is further increased when cleft is present in parents or siblings. And maternal age more than 35 years causes risk of cleft than the paternal age. Consanguinity showed 4 fold increase in clefts. Maternal diet is a prime factor as it is directly related to folic acid and vitamin supplementation apart from the socioeconomic status of family.

ABBREVIATIONS

CL- Cleft Lip

CLP - Cleft Lip Palate

CP- Cleft Palate

SES - Socioeconomic Status

NS - Non syndromic

CL/P - Cleft lip/palate

Conflicts of Interests: None

REFERENCES

[1]. Mossey P, Little J. Addressing the challenges of cleft lip and palate research in India. J Plast Surg. 2009;42(3):9-18.

[2]. Murray JC, Wehby GL, Ferreira M, Costa R, Felix T, Padovani C, et al. Oral cleft prevention programme.BMC Pediatr.2012;12:184.

[3]. Burgoon JM, Selhub J, Nadeau M, Sadler TW: Investigation of the effects of folate deficiency on embryonic development through the establishment of a folate deficient mouse model. Teratology 2002, 65(5):219-227.

[4]. Vanderas AP. Incidence of cleft lip, cleft palate, and cleft lip and palate among races: a review. Cleft Palate J. 1987; 24(3):216-225.

[5]. Ravikumar Kuppuswamy's Socio-Economic Status Scale-a revision of economic parameter for 2012. International Journal of Research & Development of Health.2013;1(1):2-4.

[6]. Czeizel AE, Dobo M, Vargha P. Hungarian cohort-controlled trial of periconceptional multivitamin supplementation shows a reduction in certain congenital abnormalities. Birth Defects Res A Clin Mol Teratol. 2004;70(11):853–861.

[7]. Van Rooij IA, Ocke MC, Straatman H, Zielhuis GA, Merkus HM, Steegers-Theunissen RP. Periconceptional folate intake by supplement and food reduces the risk of nonsyndromic cleft lip with or without cleft palate. Prev Med. 2004;39(4):689–694.

[8]. Wilcox A, Lie RT, Solvoll K, Taylor J, McConnaughey DR et al. Folic acid supplements and risk of facial clefts: national population based case-control study. BMJ. 2007;334(7591):464.epub

[9]. Jahanbin A, Shadkam E, Miri HH, Shirazi A S, Abtahi M. Maternal folic acid supplementation and the risk of oral clefts in offspring. J Craniofac Surg. 2018:29(6); e 534-e 541.

[10]. Xiao WL, Wu M, Shi B. Folic acid rivals methylenetetrahydrofolate reductase (MTHFR) gene-silencing effect on MEPM cell proliferation and apoptosis. Mol Cell Biochem. 2006;292(1-2):145-154.

[11]. Rosenquist TH, Ratashak SA, Selhub J. Homocysteine induces congenital defects of the heart and neural tube: effect of folic acid. Proc Natl Acad Sci U S A 1996;93(26):15227–32.

[12]. Knott L, Hartridge T, Brown NL, Mansell JP, Sandy JR: Homocysteine oxidation and apoptosis: a potential cause of cleft palate. In Vitro Cell. Dev. Biol. Anim 2003; 39(1-2): 98– 105.

[13]. Kelley D, Dowd T, Reulbach U. Use of folic acid supplements and risk of cleft lip and palate in infants: a population-based cohort study. Br J Gen Pract. 2012; 62(600): e466–e472.

[14]. Sperber GH. Formation of the primary palate. In: Wyszynski DF, editor. Cleft Lip and Palate: From Origin to Treatment. Oxford University Press; 2002. pp. 5–13.

[15]. Brauer PR, Tierney BJ. Consequences of elevated homocysteine during embryonic development and possible modes of action. Curr Pharm Des.2004; 10(22):2719–2732.

[16]. Gonzalez BS, Lopez ML,Rico MA, Garduno F. Oral clefts: a retrospective study of prevalence and predisposal factors in the state of Mexico. Journal of Oral Science.2008;50(2):123-129.

[17]. Betty AJ, Varsha M, Subramani SA, Shashirekha M, Jayanthi KS et al. Parental pesticidal exposure and risk of clefts in the craniofacial region: A case-control study in South India. Int J of applied biology and pharmaceutical technology. 2015; 6(2):230-235.

[18]. Kingston HM. ABC of Clinical Genetics . 3rd ed. London: BMJ;2002.P.7.

[19]. Betty AJ, Subramani SA, Varsha M, Mini J. Consanguinity and clefts in the craniofacial region: A retrospective case- control study. Journal of cleft lip palate and craniofacial anomalies. 2015;2(2):113-117.

[20]. Nouri MA, Hamad SA, Rasheed NE. Incidence of Cleft Lip and Palate in Erbil City. 2010; 7(1): 106-112.

[21]. Ravichandran K, Shoukri M, Aljohar A, Shazia NS, Al-Twaijri Y, Al Jarba I. Consanguinity and occurrence of cleft lip/palate: a hospital-based registry study in Riyadh. Am J Med Genet A. 2012;158A(3):541-546.

[22]. Ajit Parihar , Sandeep Kumar, Hu Weihsin, Sarita Parihar, Dolly Patel. Hospital linked case control community study for etiologic factors related to non-syndromic cleft lip and palate cases in Gujarat, India. Indian J. Prev. Soc. Med.2009;40 (1 & 2):77-82.

[23]. Figueiredo R F, Figueiredo N, Feguri A, Bieski I, Mello R,Espinosa M et al. The role of the folic acid to the prevention of orofacial cleft: an epidemiological study. Oral Dis.2015;21(2):240-247.

[24]. Omo- Aghoja V W, Omo- Aghoja L, Ugboko V I, Obukewe O N, Saheeb B D O,Feyi-Wabsoo P, et al. Antenatal determinants of orofacial clefts in Southern Nigeria. African Health Sciences:2010;10(1):31-39.

[25]. Farhadian N, Shahorkhi Z. Incidence rate and risk factors for cleft lip and palate hospital centres of Hamadan province, Iran in 2007. Iranian Journal of Orthodontics.2013;8:57-65.

[26]. Xingguang Zhang, Su Li, Siqintuya Wu, Xiaojin Hao, Shuyi Guo et al. Prevalence of birth defects and risk-factor analysis from a population-based survey in Inner Mongolia, China. BMC Pediatrics. 2012;12:125.

[27]. Lieff S, Olshan AF, Werler M, Strauss RP, Smith J, Mitchell A. Maternal cigarette smoking during pregnancy and risk of oral clefts in newborns. Am J Epidemiol. 1999;150(7):683–94.

[28]. Shahrukh Hashmi S, Gallaway MS, Waller DK, Langlois PH, Hecht JT. National Birth Defects Prevention Study. Maternal fever during early pregnancy and the risk of oral clefts. Birth Defects Res A Clin Mol Teratol. 2010;288(3):186–194.

[29]. Rahimov F, Juggesur A, Murray J.C. Genetics of nonsyndromic orofacial clefts. Cleft palate craniofac.J 2012;49(1):73-91

[30]. Lie RT, Wilcox AJ, Taylor J, Gjessing HK, Saugstad OD et al. Maternal smoking and oral clefts: the role of detoxification pathway genes. Epidemiology. 2008; 19(4):606– 615.

[31]. Prerana A, Biswarup B, Parthapratim G, Asis K D. Effect of socioeconomic status on clefts of lip, alveolus and palate in population belonging to the eastern part of india. National Journal of Medical Research.2014;4(3):222-224.

[32]. Dvivedi J, Dvivedi S. A clinical and demographic profile of the cleft lip and palate in Sub-Himalayan India: A hospital-based study. Indian J Plast Surgery. 2012; 45(1):115–120.

How to cite this article:

Betty Anna Jose, Varsha Mokhasi, S A Subramani, M Shashirekha. NON-GENETIC FACTORS CONTRIBUTE TO THE INCIDENCE OF NON SYNDROMIC CLEFTS IN THE CRANIOFACIAL REGION. Int J Anat Res 2019;7(3.2):6851-6858. **DOI:** 10.16965/ijar.2019.241