*Available online at* www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.7593

**ISSN: 2320 – 7051**

*Int. J. Pure App. Biosci.* **7 (3):** 625-631 (2019)

*Research Article*

# A Study on Non Syndromic Cleft Lip /or Cleft Palate on the Basis of Certain Contributory Association of Genetic Mutation / Polymorphism of Transforming Growth Factor (TGFα) & Its Relationship with Phenotypic Characteristics of the Disease

**Seema Rekha Devi[1], K. L. Talukdar[2] and Monalisa B. Deka[3*]**

[1]Professor, Department of Plastic Surgery, [2]Professor, Department of Anatomy, [3]Scientist, HLA Lab, Gauhati Medical College & Hospital, Guwahati-32

*Corresponding Author E-mail: monalisabora12deka@gmail.com

Received: 15.05.2019 | Revised: 21.06.2019 | Accepted: 27.06.2019

**ABSTRACT**

*Development of the face takes place in early uterine life between 4-8 weeks which involves interaction of several cell populations and coordination, influenced by environmental factors and genetic pathways. Disruption of the cell signaling pathways with effects of environmental insults at any stage of early development may result in myriads of Syndromic or Nonsyndromic clefts in various forms as isolated or a combination of complex deformities Non Syndromic Cleft Lip is a frequent malformation of the facial region. Genetic variants with 36 patients of CL/P and 71 parents were enrolled in this study with written informed consent following ethical clearance from ethics committee of Gauhati Medical College. Family history of the patients were collected and were found that none of the patients had a positive family history of cleft Lip or palate. The study showed that group of cleft patients, maternal blood glucose levels, Vit B12 and Serum Folate levels were looked into, to see overall deficiency / excess in the study group in general, which may have influenced during the early pregnancy. 16 mothers using biomarkers were studied for serum folate level, B12 and type 2diabetes and birth weight of the child were recorded. Birth weight was found to be reasonably low in more than half of our patients. Studies using random blood glucose levels in the maternal blood and folate serum samples were within normal limits 88-110 mg/dl and 2.14ng/mL – 3.51 ng/Ml respectively. Study also suggests that Folate- folic acid deficiency may have a role in the origin of isolated cleft lip and/or cleft palate and it can be neutralized by the supplementation of Folic acid during the critical period of these two kinds of orofacial clefts. In order to assess the association of alleles for candidate gene TGFα with non-syndromic cleft lip and palate, DNA samples from 47 patients attending Gauhati Medical College and Hospital including parents were analyzed. TGFα TaqI gene has two alleles namely allele 1 and allele 2.*

***Key words:*** *Syndromic and Non- Syndromic Cleft lip & palate, genetics, orofacial clefts, environmental* factors, *Transforming Growth factor Alpha.*

**Cite this article:** Devi, S.R., Talukdar, K.L. and Deka, M.B., A Study on Non Syndromic Cleft Lip /or Cleft Palate on the Basis of Certain Contributory Association of Genetic Mutation / Polymorphism of Transforming Growth Factor (TGFα) & Its Relationship with Phenotypic Characteristics of the Disease., *Int. J. Pure App. Biosci.* **7(3):** 625-631 (2019). doi: http://dx.doi.org/10.18782/2320-7051.7593

## INTRODUCTION

Cleft lip (cheiloschisis)/cleft palate (palatoschisis), is one of the commonest congenital anomaly occurring in 1: 750 live births. In Indian subcontinent, one of the most populous country in the world with a population of (2019 latest UN data), the approximate population is 1, 350, 438098 one can imagine cleft prevalence in our country. It is estimated birth prevalence of clefts is somewhere between 27000-33000 clefts per year[1]. In 2008, the World Health Organization (WHO) has recognized that non-communicable diseases including birth defects cause significant infant mortality and childhood morbidity and have included Cleft Lip & Palate in their Global burden of disease (GBD) initiative[1].

Non Syndromic clefts may present as isolated Cleft Lip and/or palate in various forms from microform & incomplete variety to complete representation of spectrum of deformities, caused by abnormal facial development during gestation. Overall global prevalence of Orofacial cleft is one in 600 new born babies. Assuming 15,000 births per hour worldwide (US Bureau of the census, 2001) a child is born with a cleft somewhere in the world approximately every 2 minutes. Despite efforts to record the frequency of birth defects over the years, accurate data on the epidemiology do not exist in many countries including India[2].

Non syndromic Cleft lip & palate is a multifactorial disorder involving both genetic & environmental factors which contributes to its aetiology.

Individuals with cleft lip/palate (CLP) feeding problems, recurrent middle ear infection[3]. As the individual continues to grow defects in tooth development and require dental or sometimes surgical treatment.

Involvement of lifestyle and environmental factors and its interaction with genetic factors with implication of various genes has been the subject of research without any definite outcome and it is still illusive as to which particular Genetic mutation and or polymorphism is responsible for such birth defects. Characterization of genomic sequences will greatly impact regulation of Gene networks and pinpoint any variation s in different stages of craniofacial morphologies[3] Various studies have been made to understand the etiology of this disorder so as to predict its occurrence and to prevent it.

Advances in molecular and genetics biology have begun to reveal the basis of craniofacial development and to identify the genes associated with CLP[4]. There are studies which reviews to explore the possibility of association of key genes like TGFß, GABRB3 through PCR-sequencing methods, and provides an update on the etiological factors underlying this common malformation[5].

Clinically, when CLP appears with other (usually two or more) malformations in recognizable patterns, it is classified as syndromic CLP (SCLP). If it appears as an isolated defect or if syndromes cannot be identified, the term non syndromic CLP (NSCLP) is used. The number of CLP syndromes is large and still growing.

## MATERIALS AND METHODS

The study was conducted in the Department of Plastic Surgery, Gauhati Medical College and Hospital, Guwahati, Assam from September 2013 to February 2019. Blood samples were collected from patients with proper consent and documentation was done on the basis of history and clinical data. Also the general health status of every individual and their environmental background were recorded. DNA patterns of promoter regions were analyzed for specific genes implicated in CL/P focusing TGFα followed after Poncz *et al.*[6] PCR for molecular study were performed using Ampli Taq DNA polymerase (Life technologies, USA) and the primer sequences were used for PCR reaction are shown in Table 1. DNA was extracted from whole blood using QIA amp DNA Mini kit (Qiagen GmbH, Hildan). DNA was used for PCR analysis the same day and an aliquot was stored at - 80°C for future use.

**Table 1: Primer sequences used for PCR amplification**

| Gene name | Forward primer (5′ – 3′) | Reverse primer (5′ - 3′) | Product size (bp) |
|---|---|---|---|
| TGF-α | TCA CTT CCC CTT TTT CAT CTG | CGA GGA GGC TCT GAG GTG | 174/178 [9] |

## RESULTS AND DISCUSSION

Descriptive characteristics of parents and infants were recorded and interestingly, it was found that none of the patients had a positive family history of cleft Lip or palate. Most people in the study group belonged to the poor socio economic group leading to a stressful life; however there most of the case recorded was found to be female. There were no differences in maternal age between the cases.

In our study group of cleft patients, maternal blood glucose levels, Vit B12 and Serum Folate levels were looked into, to see overall deficiency / excess in the study group in general, which may have cause influence during the early pregnancy[7]. With the aim to study the biomarkers, 16 mothers were randomly selected for the study for serum folate level, B12 and type 2 diabetes and birth weight of the child were recorded . Serum B6 level was not estimated, as this test was not available in our institution. Birth weight was found to be reasonably low in more than half of our patients. Vitamin B12 estimation in the 16 mothers were between 463.6 pg/ml-568 pg/ml (normal range 156-663 pg/ml) within normal limits and may not impact upon the incidence of cleft palate. However findings of[8] Zhao *et al.*[8] and[9] Tanabe *et al.*[9] suggest that VitB12 may inhibit the expression of some cleft palate inducers.

The study showed that the Serum Folate level was within low to intermediate levels (2.14ng/mL – 3.51ng/Ml) which is justified by the findings of[7] Sutton *et al.*[7] as the Homo cysteine levels in early pregnancy are not associated with all congenital malformations excluding NTDs (Neural tube defects). However finding of[10] Czeizel *et al.*[10] suggests that Folate-folic acid deficiency may have a role in the origin of isolated cleft lip and/or cleft palate and can be neutralized by the supplementation of Folic acid during the critical period of these two kinds of oro-facial clefts[11].

DNA sequencing of the amplified PCR product was carried out in ABI 3730 x l instrument. Same set of primers as used for PCR were also used for sequencing. Sequencing was carried out for the patients and their parent. Sequence data generated were analyzed with the following softwares. Bio Edit sequence analysis editor, ver 5.0.9 software for analyzing sequence electro phero gram for resolving nucleotide ambiguities (Fig 1). Genedoc multiple sequence alignment editor & sharing utility version 2.1.02, was used for sequences editing alignment of DNA sequences (Fig 2).

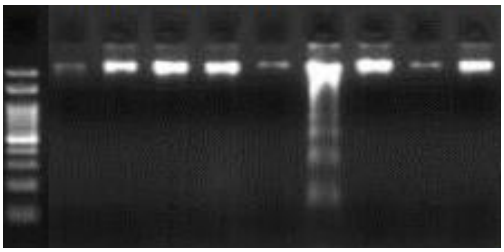
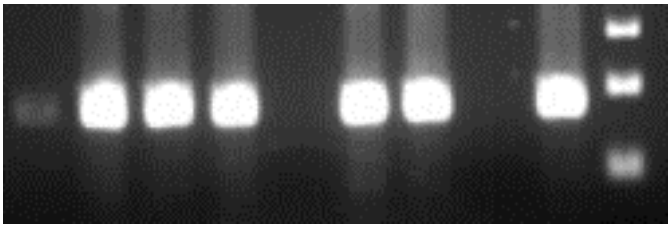


**Fig. 1: 0.8% agarose gel showing genomic DNA**



178bp

**Fig. 2: 1.5% agarose gel showing PCR amplified product for TGF α**

Online NCBI BLAST program (www.ncbi.nlm.nih.gov/blast) was used to compare sequence generated from this study with the existing sequences available on NCBI (Fig 3).

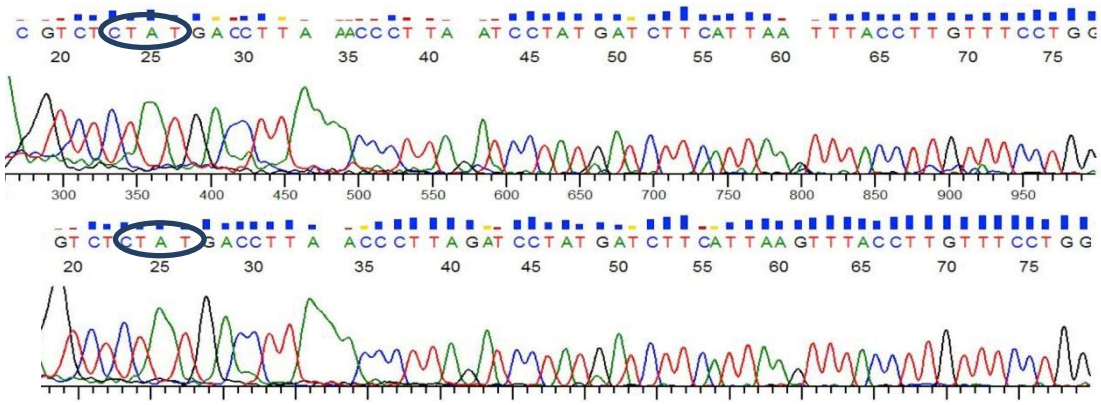


**Fig. 3: Representative sequence chromatograms of TGFα*Taq1* gene. Allele 1 was detected in all cases**

Two known alleles for the TGFα *Taq*1 gene were identified and were found to be in accordance with Ardinger *et al.*[12]. Allele 1 gives a 178 bp PCR product where as in allele 2 four nucleotides are missing (Fig 4).

Allele 1

**TCACTTCCCCTTTTTCATCTGT**AAAAGGAGGAATTTGGCCTATGA
AAGGTCTCTAATGACCTTAAAACCCTTAGATCCTATGATCTTCATTAAGTTTACCTTG
TTTCCTGGATATTTTCGCCAACATCCATGAACACATCAGGATGTGGGGCCCAGCT TG
CACCT **CAGAGCCTCC TCG**

Allele 2

**TCACTTCCCCTTTTTCATCTGT**AAAAGGAGGAATTGGCCTATGAAAGGTCTC- - - -
GACCTTAAAACCCTTAGATCCTATGATC
TTCATTAAGTTTACCTTGTTTCCTGGATATTTTCGCCAACATCCATGAACACATCAGGAT**G**
**TGGGGCCCA GCTTGCACCT CAGAGCCTCC TCG**

**Fig 4: TaqI-primer allele sequence. Allele 2 lacks 4 nucleotides as shown. Primer sequences are in bold**

Gene environment causes of Cleft Lip & Palate and its contributions to Non Syndromic forms of clefting and their implications for developmental biology has been studied by Murray *et al*[13].

Protective effect of Vit B12 on palatal developmental deficit has been studied in Mice and suggested that Vit B12 may not impact the incidence of Cleft Palate. However it affects the expressions of TGF-B3 and ALK 5 in TCDD+ DEX exposure induced cleft palate. Shu-fan Zhao *et al.*[14], this result was similar to the present study where no significant impact of Vit B12 deficiency was seen.

B group of vitamins including folic acid supplementation during pregnancy have been shown to be effective in preventing Cleft Lip and Palate (CLP) in humans. The clinical trials for the prevention of malformation have been mostly empirically based. It was suggested that a deficiency of each of the individual B vitamins is teratogenic however total B group deficiency has the strongest in the case of deficiency of deficiency of all B vitamins. They concluded that the results may help to elucidate the interplay of genetic conditions and exogenons (nutritional) factors in both the aetiology and prevention of CLP.[15]

There are studies which supports the role for TGFA in craniofacial morphogenesis and support an interrelated mechanism underlying non syndromic forms of cleft lip and palate. Genetic analysis and tissue expression studies have confirmed an association of alleles of TGFA with non syndromic cleft lip with or without cleft palate (CL/P) in humans. A significant association between alleles of TGFA & CPO was identified which supports a role for this gene as one of the genetic determinants of craniofacial development. Sequence analysis of the variants disclosed a cluster of three variable sites within 3 bp. Of each other in the 3 untranslated region previously associated with an antisense transcript. These studies extend the role for TGFA in craniofacial morphogenesis and support an inter-related mechanism underlying non-syndromic forms of cleft lip and palate[16]. The present study revealed that two known alleles for the TGFα *Taq1* gene were identified and were found. Allele 1 gives a 178 bp PCR product where as in allele 2 four nucleotides are missing.

Similar study was carried out by Anil Melani Maskoen *et al*[17] in Indonesian population concluded that TGFA Rsal gene can be considered a risk factor of Non syndromic CPO COMPARED TGFA Bam HI gene of Indonesian subjects. In this case control study using samples from 32 NS CPO subjects and 28 control subjects. DNA was extracted from venous blood and the TGFA gene was amplified using polymerase chain reaction technique, then digestion product from Taql and Rsal restriction enzyme was evaluated. Variants of TGFA which include three common poly morphism of gene variants of TGFA gene (TGFA Rsal TGFA Taql in intron 5, and TGFA Bam HI in exon[17].

In another case control study, 113 children with NSCLP and 209 controls were included. Genotyping of the Taql polymorphism was performed by Polymerase chain reaction and restriction fragment lenth polymorphism methods, A p- value of 0.05 was considered statistically significant[18].

There has been marked progress in identifying genetic and environmental triggers for Syndromic CLP, the aetiology of more common Non-Syndromic (isolated) forms remains poorly characterized. Using a combination of Epidemiology, careful phenotypic Genome – wide association studies and analysis of animal models, several distinct Genetic and Environmental factors have been identified and confirmed for non syndromic CLP. Maternal smoking & exposure to maternal alcohol consumpsion, besides nutrients & toxins, other environmental exposures have been assessed to have possible roles in Clefting[19].

## CONCLUSION

The study conducted shows that patients suffering from non syndromic cleft lip and palate are for socio economic backward classes. Most of the patients are found to be female. Results related to Vitamin B12 and Serum Folate were between normal range whereas the birth weight was found to be reasonably low in more than half of our patients and may not impact upon the incidence of cleft palate. Sequence results confirmed that all the sequenced samples carried homozygous TGFα *Taq*1 allele however much lower sample size of the sequenced data limits the study necessitating sequencing the rest of the samples. As such, high volume of cases in the state of Assam and other North Eastern states in India with a diverse group of population, it is important to study the genotype and phenotype co-relation and genetic polymorphism, and a gene environment study in a larger group of patients to establish a definitive evidence of gene – gene & gene- environment interactions. With ever increasing birth defects, it will remain a challenge unless we have a better understanding of the cell signalling pathways and environment and genetic interactions. The study opens a chapter for exploring the possibility of association of other key genes like TGFß, GABRB3 through PCR-sequencing methods in future.

**Acknowledgment**

The authors would like to acknowledge Indian Council of Medical Research (ICMR) for funding the Research work.

**REFERENCES**

1. Mossey, P. A., Little, J., Addressing the challenges of cleft lip and palate research in India. *Indian Journal of Plastic Surgery*, **42:** supplement 1, S9-S18 (Sept 2009).
2. Mossey, P. A., Little, J., Chapter 12: Epidemiology of oral clefts: an international perspective In: Wyszynski, D. F., editor Cleft lip and palate. From origin to treatment. Oxford University Press ISBN: 0-19-513906- 2. P. 127-58 (August 2002).
3. Janaki, C. S. and Lokanadham, S., A review on candidate genes or Loci and their importance in non syndromic cleft lip and cleft palate. *J Genet DNA Res*. **2(1):** 107 (2018).
4. Feinberg, A. P. and Vogelstein, B., A technique for radio labeling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem*. **132:** 6 – 13 (1983).
5. Dixon, M. J., Garner, J. and Ferguson, M. W. J., Immunolocalisation of epidermal growth factor (EGF), EGF receptor and transforming growth alpha (TGFa) during murine palatogenesis in vitro and in vivo. *Anat Embryol*. **184:** 83–91 (1991).
6. Poncz, M., Solowiejczyk, D., Harpel, B., Mory, Y., Schwartz, E. and Surrey, S., Construction of humen gene libraries from small amounts of peripheral blood: analysis of β-like globin genes. *Hemoglobin*. **6:** 27 – 36 (1982).
7. Sutton, M., Mills, J. L., Molloy, A. M., Troendle, J. F., Brody, L. C., Conley, M., Mc Donnell, R., Scott, J. M. and Kirke, P. N., Maternal folate, vitamin B12 and homocysteine levels in pregnancies affected by congenital malformations other than neural tube defects. *Birth Defects Research Part A: Clinical and Molecular Teratology*. **91(7):** 610–615 (2011).
8. Zhao, S. F., Chai, M. Z., Wu, M., He, Y., Meng, T. and Shi, B., Effect of vitamin B12 on cleft palate induced by 2, 3, 7, 8 - tetrachlorodibenzo-p-dioxin and dexamethasone in mice. *Univ-Sci B (Biomed & Biotechnol)*. **15(3):** 289-294 (2014).
9. Tanabe, A., Taketani, S., Endo-Ichikawa, Y., Tokunaga, R., Ogawa, Y. and Hiramoto, M., Analysis of the candidate genes responsible for non-syndromic cleft lip and palate in Japanese people. *Clin Sci*. **99(2):** 105-11 (2000).
10. Czeizel, A. E. and Sárközi, T. L., Dose-dependent effect of folic acid on the prevention of orofacial clefts. *Pediatrics*. **104(6):** 66 (1999).
11. Hwang, S. J., Beaty, T. H., Panny, S. R., Street, N. A., Joseph, J. M., Gordon, S., McIntosh, I. and Francoman, C. A., Association study of transforming growth factor alpha Taq 1 polymorphism and oral clefts: indication of gene–environment interaction in a population-based sample of infants with birth defect. *Am J Epidemiol*. **141:** 629–636 (1995).
12. Ardinger, H. H., Buetow, K. H., Bell, G. I., Bardach, J., Van Demark, D. R. and Murray, J. C., Association of genetic variation of the transforming growth factor alpha gene with cleft lip and palate. *Am J Hum Genet*. **45:** 348–353. defects. *Birth Defects Research Part A: Clinical and Molecular Teratology*. **91(7):** 610–615 (1989).
13. Gene/environment causes of Cleft Lip or Palate – NCBI- NIH. https//www.ncbi.nim.nih.gov.pubmed. by J C Murray (2002).
14. Effect of Vit B12 on Cleft Palate induced by 2, 3, 7, 8 - tetrachlorodibenzo -p-dioxin and dexamethasone in mice. Shu-fan Zhao, Mao-Zhaou Chai, (…) and Bing-Shi https//www.ncbi.nim.nih.gov articles.
15. B group vitamins and Cleft Lip and Palate. Schubert J, *International Journal of Oral Maxillofacial Surgery*. (2002).

16. Association of Transforming growth factor alpha gene polymorphism with nonsyndromic cleft palate only (CPO) American Journal of Human Genetics **53(4):** 836-43. Rita Shiang, A. C., Lidral, H. H., Ardinger, K. A., Buetow, Paul, A., Romitti, Ronald Munger, Jaffrey C Murray (Nov 1993).
17. Expression of transforming growth factor alpha Bam HI and Rsal gene variants associated with non-syndromic cleft palate of Indonesian subjects using polymerase chain reaction method. *Asian Journal of Pharmaceutical & Clinical research* **11(4):** 351 Anil Mellllani Maskoen (April 2018).
18. Association study of Transforming Growth Factor Alpha Taql polymorphism and the risk of Cleft Lip and/ or palate in an Iranian population: TGFA Taql polymorphism and the risk of CLP. HTTPS//DOI.ORG/10.1002/BDR2.1125 Fahimch Bagheri (Oct 2017).
19. Cleft Lip and Palate: Synthesizing genetic & environmental influences. Michael J. Dixon, Mary L. Marazita (.) and Jeffrey C. Murray. https//www.ncbi.nim.nih.gov