

## Homocysteine Level- A Biomarker in individuals having non-syndromic cleft lip with or without palate

Dr. Irshad Ahmed<sup>#\*</sup>, Dr. Afreen Nadaf (MDS, Consultant), Dr. Aamira (BDS, Dental Surgeon) and Dr. Masroor Shah (BDS, Dental Surgeon)

<sup>#</sup>Associate Professor, Oral and Maxillofacial Surgery, GDC, Srinagar, India

Received 10 Oct 2019, Accepted 11 Dec 2019, Available online 13 Dec 2019, Vol.7 (Nov/Dec 2019 issue)

### Abstract

**Introduction:** Cleft lip with or without cleft palate (CL/P) is one of the most common birth defects worldwide and is characterized by abnormalities of the orofacial structure. NSCL  $\pm$  P has a multifactorial etiology including abnormal homocysteine (Hcy) level and anomalous developmental end points which is not well understood.

**Aim:** The aim of this study was to evaluate the Hcy levels in NSCL  $\pm$  P patients.

**Materials and Methods:** This study was carried out under Smile train center of Khyber medical institute, 20 patients and 20 controls participated in the study. Blood samples were collected from both the patients and controls and assessed for serum Hcy level using chemiluminescent immunoassay technique. Student's t-test was used for statistical analysis.

**Results:** The average Hcy concentration was 8.6  $\mu$ mol/L in control group. There was an increase in Hcy concentration among the NSCL  $\pm$  P cases with an average value of 19.4  $\mu$ mol/L. The results were found to be statistically significant using Student's t-test.

**Conclusions:** Our results showed that Hcy concentration was elevated in NSCL  $\pm$  P patients when compared with that of controls.

**Keywords:** Chemiluminescent immunoassay, homocysteine, nonsyndromic cleft lip with or without palate, orofacial clefts

### Background

Cleft lip with or without cleft palate (CL/P) is one of the most common birth defects worldwide and is characterized by abnormalities of the orofacial structure.<sup>1</sup> The prevalence of CL/P among newborns is approximately 1/700.<sup>2</sup> The overall worldwide prevalence of the cleft lip with or without the cleft palate was 9.92 per 10,000. The prevalence of the cleft lip was 3.28 per 10,000, and that of the cleft lip and palate was 6.64 per 10,000.<sup>3</sup> Birth prevalence of clefts is somewhere between 27,000 and 33,000 clefts per year.<sup>4</sup> Lowest incidence occurs in Native

American tribes of Montana, USA, which is 1:2076.<sup>5,6</sup> Approximately 30% of CL/P cases are syndromic. However, at least 70% of CL/P cases are nonsyndromic, characterized by isolated orofacial cleft without any known syndrome. Unlike syndromic CL/P, the etiology underlying nonsyndromic CL/P (NSCL/P) remains less understood. Nonetheless, it is believed that NSCL/P is a complex disorder caused by both genetic and environmental factors, as well as their underlying

interactions. To date, both genome-wide and candidate gene approaches have been used to decipher the genetic etiology underlying NSCL/P. In contrast to genome-wide approaches such as genome-wide association studies (GWASs) and linkage analysis, the candidate genes approach is based on prior knowledge of NSCL/P pathogenesis.<sup>2</sup>

NSCL  $\pm$  P has a multifactorial etiology including abnormal homocysteine (Hcy) level and anomalous developmental end points which is not well understood.<sup>7</sup> The other etiological factors include heredity, consanguinity, maternal environment and demographic factors, and aspects such as intrauterine posture, drugs, vitamins, alcohol consumption, smoking, infections and diet.<sup>8,9</sup>

Previous studies have reported that folic acid supplementation decreased the risk of NSCL/P during early pregnancy. It is widely believed that the occurrence of NSCL/P is related not only to folic acid deficiency but also to hyperhomocysteinemia (Hcy) caused by a genetic defect in the folate metabolism pathway. Evidence shows that Hcy is associated with a high risk of NSCL/P incidence.<sup>10</sup> Elevation in the level of blood homocysteine (Hcy) and polymorphisms/mutations in the Hcy-pathway

\*Corresponding author's ORCID ID: 0000-0003-3206-6330

DOI: <https://doi.org/10.14741/ijmcr/v.7.6.17>

genes that lead to its elevation have been identified as risk factors for certain other congenital (e.g. NTD) and late-age disorders (e.g. cardiovascular, cancers). This association differs from region to region and between different populations.<sup>11</sup>

Hcy is a sulfur-containing amino acid present in the body which does not take part in the formation of proteins.<sup>12</sup> It is formed as an intermediate in the methionine pathway.<sup>13</sup> Hcy, under normal conditions, is converted to either methionine by remethylation or to cystathionine through trans-sulfuration pathway.<sup>14</sup> The remethylation reaction catalyzed by the enzyme methionine synthase (MS) requires folate and vitamin B12 (or betaine in another type of reaction) as cofactors whereas the trans-sulfuration reaction is catalyzed by cystathionine-β-synthase (CBS) using pyridoxal-5'-phosphate (coenzyme form of vitamin B6) as a cofactor. S-adenosylmethionine allosterically inhibits MTHFR and also activates CBS, thus coordinating the two pathways.<sup>9,14</sup>

MTHFR gene regulates the concentration of Hcy in blood by causing demethylation of 5-methyltetrahydrofolate to tetrahydrofolate and hence forming methionine from Hcy in the process.<sup>15</sup> Any error in the molecular composition of MTHFR gene sequence or gene regulatory mechanism might result in the accumulation of Hcy in blood which might have a role in the formation of orofacial clefts.

Thus the present study was undertaken with the aim of investigating serum Hcy as a risk factor for NSCL ± P in Kashmiri population.

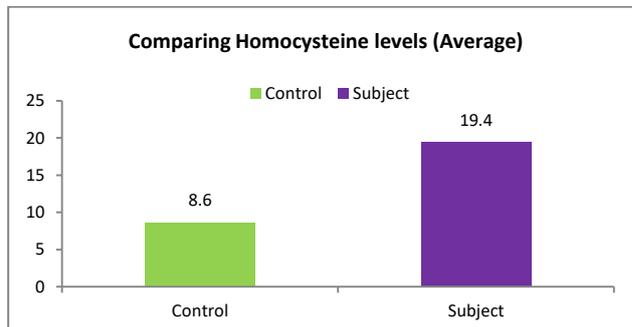
**Materials and Methods**

The study participants included patients enrolled under Smile train center of Khyber Medical Institute, Srinagar. A total of 40 participants were considered, of which 20 were grouped as controls and 20 were grouped as patients. Control group comprised of participants with no systemic abnormalities and orofacial deformities. The patient group comprised of participants having NSCL ± P within the age group of 1-5 years. Condition/diseases where Hcy levels were elevated such as congenital vascular diseases, neurodegenerative and psychiatric conditions were excluded in both patient group and control group. Ethical clearance was obtained for the study from the Institutional Ethical Clearance Committee. Blood samples were collected separately from both control and patient groups and assessed for the concentration of Hcy from serum using competent chemiluminescent immunoassay technology. Student's t-test was used to analyze the values of Hcy concentrations obtained in blood samples of both control and patient groups.

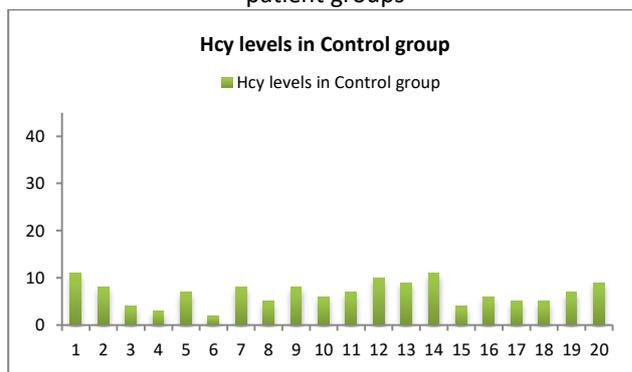
**Results**

Significant Mean Hcy level differences were recorded among control group (8.6 ± 1.23µmol/L) and patient

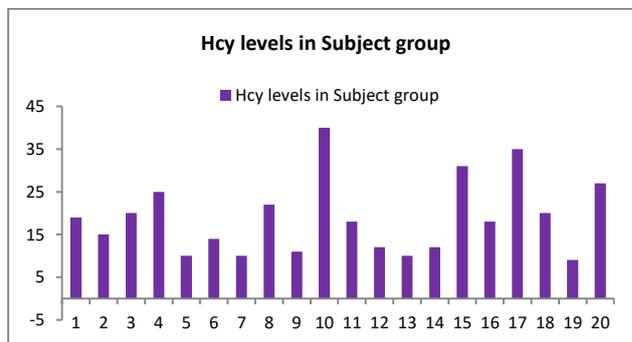
group (19.4 ± 5.6 µmol/L) [Figure 1]. Control group comprising individuals without CL/P had relatively moderate level of Hcy in their serum samples [Figure 2] whereas patient group with NSCL ± P had elevated level of Hcy [Figure 3].



**Figure 1:** Comparing homocysteine levels in control and patient groups



**Figure 2:** Homocysteine concentration of participants in control group



**Figure 3:** Homocysteine concentration of participants in Subject group

**Discussion**

The normal homocysteine level in the blood is between 4 and 15 micromoles/liter (µmol/L). Any measurement above 15 is considered high. Any measurement below 12 is considered low. Optimal homocysteine levels are below 10 to 12. Hyperhomocysteinemia has been classified into moderate, intermediate, and severe types based on the level of homocysteine and are: moderate (15 to 30

μmol/L), intermediate (30 to 100 μmol/L), severe (greater than 100 μmol/L). The average Hcy level among healthy Indian population is reported to be 12.5 μmol/L. In this study, the average Hcy level was recorded as  $8.6 \pm 1.23$  μmol/L (mean  $\pm$  standard deviation) in control group and  $19.4 \pm 5.6$  μmol/L in patient group, indicating a possibility of the accumulation of Hcy in blood. Hence, it can be implied that there is a clear increase in the concentration of Hcy in cases having NSCL  $\pm$  P.

Past studies have identified some variables associated with cleft lip and/or palate. Vitamin supplements during pregnancy (especially folic acid with or without vitamins) appear to play an important role in non-syndromic cleft lip and/or palate prevention; other micronutrients have also been implicated as protective factors in orofacial clefts such as B1, B6, myo-inositol, zinc, iron and riboflavin.<sup>16-19</sup>

Folate is a one-carbon donor; as such, it is involved in the biosynthesis of purines and pyrimidines and in homocysteine remethylation, which produces methyl groups for methylation of DNA, proteins, and lipids. Whether folate can regulate directly gene expression is unknown, although several vitamins are known to do so by interacting with a nuclear receptors family of transcription factors, for example, retinoids and vitamin D (35). Folate may also regulate the expression of several essential genes for cellular multiplication and differentiation during embryogenesis, especially those involved in palate and lip formation.<sup>19</sup>

Many factors contribute to high homocysteine levels- deficiency of folate or B vitamin in diet, other risk factors include: low thyroid hormone levels, psoriasis, kidney disease, certain medications, genetics. The MTHFR gene produces an enzyme that helps regulate homocysteine levels in the body. If there is a mutation in the MTHFR gene, homocysteine levels may not be regulated properly. Genetic mutations in MTHFR are the most commonly known inherited risk factor for elevated homocysteine levels.<sup>20</sup>

Previous studies have showed a preventive effect against NSCL  $\pm$  P of increasing food folate intake in the preconceptional period. Evidence also suggests that deficiencies of myo-inositol and zinc are involved in reproductive failures, in particular, in midline defects as neural tube defects. NSCL  $\pm$  P and neural tube defects both originate from neural crest cells.<sup>21</sup>

An inadequate amount of zinc is teratogenic in animals and humans. Being a co-factor for several enzymes and a constituent of various proteins, it is crucial for embryonic development.<sup>18</sup> Zinc finger proteins are important in their control of genes involved in embryonic development and their mutations may exhibit several congenital malformations including cleft palates.<sup>19</sup> It also has a role in the absorption of natural folate (e.g. polyglutamates), its involvement in the conversion of 5-methyltetrahydrofolate into tetrahydrofolate by the zinc-dependent methionine synthase enzyme which regulates the amount of Hcy in blood.<sup>21</sup>

It has been shown that Hcy enters the fetus through amniotic fluid and induces apoptosis of the palatal mesenchyme that prevents fusion of the palate.<sup>22</sup> Therefore reduced amounts of zinc and folate in the metabolic system can be directly correlated with the accumulation of Hcy in the blood. Thus Hcy in the blood might act as an important biochemical feature of developmental anomalies in the orofacial region especially cleft lip and palate.

## Conclusion

NSCL  $\pm$  P is a complex congenital malformation presenting both clinical and genetic heterogeneity. The genetic basis of orofacial clefts remains poorly understood, and the identification of additional risk factors for NSCL  $\pm$  P beyond the few thus far known would greatly advance efforts at genetic counseling and perhaps prevention. Our study thus showed that serum Hcy assay could serve as a biomarker for cleft anomalies and supplementation of zinc and folate in the preconceptional period could prevent such anomalies.

## References

- [1]. Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21 (2), 263–265.
- [2]. Wang Y, Sun Y, Huang Y, Pan Y, Jia Z, Ma L., et al. Association study between Van der Woude Syndrome causative gene GRHL3 and nonsyndromic cleft lip with or without cleft palate in a Chinese cohort. *Gene* 588 (2016) 69–73.
- [3]. Prevalence at birth of cleft lip with or without cleft palate: Data from the International Perinatal Database of Typical Oral Clefts (IPDTC). *Cleft Palate Craniofac J* 2011;48:66-81.
- [4]. Mossey P, Little J. Addressing the challenges of cleft lip and palate research in India. *Indian J Plast Surg* 2009;42(suppl):9-18.
- [5]. 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 Surg* 2012;45:115-20.
- [6]. Smith WP. Cleft lip and Palate. In: Russell RC, Williams NS, Bulstrode CJ, editors. *Bailey and Love's Short practice of surgery*. 23rd ed. London: Arnold Publishers; 2000. p. 586-7.
- [7]. Saikrishna L, Bhaskar LV, Rajasekhar M. Molecular genetics of non-syndromic clefts revisited. *Int J Genet* 2011;3:23-34.
- [8]. Murray JC. Gene/environment causes of cleft lip and/or palate. *Clin Genet* 2002;61:248-56.
- [9]. Abdulla R, Tellis RC, Athikari R, Kudkuli J. Evaluation of homocysteine levels in individuals having nonsyndromic cleft lip with or without palate. *J Oral Maxillofac Pathol* 2016;20:390-4.
- [10]. Wei Lei, Yinlan Xia, Yang Wu, Gang Fu, and Aishu Ren. Associations Between MTR A2756G, MTRR A66G, and TCN2 C776G Polymorphisms and Risk of Nonsyndromic Cleft Lip With or Without Cleft Palate: A Meta-Analysis. *Genetic Testing And Molecular Biomarkers* 2018;22( 8):1-9.

- [11]. Kumari P, Ali A, Sukla KK, Singh SK and Raman R 2013 Lower incidence of nonsyndromic cleft lip with or without cleft palate in females: Is homocysteine a factor? *J. Biosci.* 38; 21–26.
- [12]. Blom HJ, Smulders Y. Overview of homocysteine and folate metabolism. With special references to cardiovascular disease and neural tube defects. *J Inherit Metab Dis* 2011;34:75-81.
- [13]. Rodríguez-Nieto S, Chavarría T, Martínez-Poveda B, Sánchez-Jiménez F, Rodríguez Quesada A, Medina MA. Anti-angiogenic effects of homocysteine on cultured endothelial cells. *Biochem Biophys Res Commun* 2002;293:497-500.
- [14]. Lentz SR. Mechanisms of homocysteine-induced atherothrombosis. *J Thromb Haemost* 2005;3:1646-54.
- [15]. Selhub J. Homocysteine metabolism. *Annu Rev Nutr* 1999;19:217-46.
- [16]. Wehby GL, Murray JC. Folic acid and orofacial clefts: A review of the evidence. *Oral Dis.* 2010;16:11-9.
- [17]. van Rooij IA, Vermeij-Keers C, Kluijtmans LA, Ocké MC, Zielhuis GA, Goorhuis-Brouwer SM, *et al.* Does the interaction between maternal folate intake and the methylenetetrahydrofolate reductase polymorphisms affect the risk of cleft lip with or without cleft palate? *Am J Epidemiol.* 2003;157:583-91.
- [18]. Wilcox AJ, Lie RT, Solvoll K, Taylor J, McConaughy DR, Abyholm F, *et al.* Folic acid supplements and risk of facial clefts: National population based case-control study. *BMJ.* 2007;334:464.
- [19]. Gladys Acuña-González<sup>1,2</sup>, Carlo E. Medina-Solís<sup>3</sup>, Gerardo Maupomé<sup>4,5</sup>, Mauricio Escoffie-Ramírez<sup>6</sup>, Jesús Hernández-Romano<sup>7</sup>, María de L. Márquez-Corona<sup>3</sup>, Arturo J. Islas-Márquez<sup>3</sup>, Juan J. Villalobos-Rodelo. Family history and socioeconomic risk factors for non-syndromic cleft lip and palate: A matched case-control study in a less developed country. *Biomédica* 2011;31:381-91.
- [20]. Elizabeth A. Varga; Amy C. Sturm; Caron P. Misita; Stephan Moll. Homocysteine and MTHFR Mutations. Relation to Thrombosis and Coronary Artery Disease. *Circulation.* 2005;111:e289-e293.)
- [21]. Ingrid P.C. Krapels, Iris A.L.M. van Rooij, Ron A. Wevers, Gerhard A. Zielhuis, Paul H.M. Spauwen, Wim Brussel, Régine P.M. Steegers-Theunissen. Myo-inositol, glucose and zinc status as risk factors for non-syndromic cleft lip with or without cleft palate in offspring: a case-control study. *Br J Obstet Gynaecol.* 2004;111, 661–668.
- [22]. Krapels IP, van Rooij IA, Ocké MC, van Cleef BA, Kuijpers-Jagtman AM, Steegers-Theunissen RP. Maternal dietary B Vitamin intake, other than folate, and the association with orofacial cleft in the offspring. *Eur J Nutr* 2004;43:7-14.