Soluble Human Leukocyte Antigen Molecules Detected in Orofacial Cleft Patients: A Case-Control Study

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Soluble Human Leukocyte Antigen Molecules Detected in Orofacial Cleft Patients: A Case-Control Study

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Abstract

Objective: To compare soluble HLA-C and HLA-DR molecules present in the plasma of orofacial cleft and non-orofacial cleft populations. **Material and Methods:** Orofacial cleft patients were recruited using an accidental sampling approach (n=15). Peripheral blood was collected from the participants and processed for Enzyme Linked Immunosorbent Assay (ELISA) against HLA-C and HLA-DR with specific antibodies. The absorbance was calculated utilizing ELISA reader. Data were statistically analyzed using an independent t-test to compare the disease and control groups. **Results:** The levels of soluble HLA-C and HLA-DR were significantly higher in the diseased group compared to the control group ($p<0.05$). **Conclusion:** The role of HLA molecules in non-communicable disease and congenital anomalies, particularly orofacial cleft, remains speculative despite the positive results of this study and those of previous investigations. It suggests that the variables examined may affect specific pathways involved in the pathogenesis of orofacial cleft, and predispose the individuals concerned to the oral cleft.

Keywords: Cleft Palate; Cleft Lip; HLA Antigens.

Introduction

Orofacial Cleft (OFC) or Cleft Lip and Palate (CLP) is the most common congenital defect in newborns. The cleft affects structures surrounding the oral cavity and leads to facial deformity resulting in poor esthetic appearance and functional impairment in mastication, hearing and speech [1]. It is one of the non-communicable conditions that cause significant rates of mortality and morbidity and is included in the Global Burden of Disease (GBD) initiative established by the World Health Organization (WHO) [2].

Its etiology is multifactorial, ranging from genetic to environmental. Rather than a single gene defect, the pathogenesis of OFC is influenced by several genes involved in embryonic orofacial structure development [3]. Numerous attempts have been made to analyze the predisposing genetic factors related to OFC. Several, including Human Leukocyte Antigen (HLA), remain speculative [4].

Considerable research has been conducted into major histocompatibility complex (MHC), which in humans is located in chromosome 6p21 and known as Human Leukocyte Antigen (HLA) [5]. It pervades six classical HLA genes and at least 132 protein-coding genes, executing the crucial role of regulating the immune system and involved in molecular and cellular processes [6]. HLA is categorized as class I, class II, or class III. HLA class I is expressed in nucleated cells, while HLA class II is found on the surface of antigen-presenting cells (APC) [7]. Unlike class I and II HLA that feature an MHC complex, HLA class III may contain molecules such as tumor necrosis factor (TNF), complement proteins, heat shock protein (HSP) and hydroxylase enzyme which are supportive of biological processes [8].

The major purpose of HLA molecules is to introduce peptide antigens into T lymphocyte cells in order to stimulate an adaptive immune response [9]. Approximately 40-50% of the possibility of developing pathological conditions results from genetics factors, with the remaining risk arising from harmful environmental etiologies. The variation in class I and II HLA genes accounts for approximately half of the risk of genetic disease with about 40-50 remaining genes related to such risk [10]. The initial association of the HLA gene with human disease coincided with the discovery of the relationship between HLA-B and Hodgkin lymphoma. MHC has subsequently been identified as the region of the genome that is related to a variety of human diseases [11]. HLA has been used as a biomarker and point of interest in the study of various conditions including autoimmune issues, infection, neoplasm and idiopathic diseases [8], diabetes type I [10], ankylosing spondylitis [12], Parkinson's disease [13], and HIV [14].

Biological markers, also referred to as biomarkers, have been defined as medical indicators of a normal physiological, pathogenic process or a pharmacological response to medical treatment. The marker could be evaluated through its molecular, histologic, radiographic, or physiologic features. However, a biomarker is specific to the direct examination, known as a clinical outcome assessment (COA), of how a patient feels, functions and survives. Certain types of a biomarker that elucidate the risk of developing a disease or medical condition in an individual who currently presents no evident disease is known as a susceptibility or risk biomarker [15].

HLA has been investigated as a potential susceptibility marker of various diseases, including OFC [4]. However, its clinical use remains limited and requires further investigation and development. The association between HLA and disease predominately consists of infectious and autoimmune cases [16]. The role of HLA in non-infectious and non-autoimmune diseases remain inadequately studied, despite several attempts to establish the relationship between HLA and developmental defects such as neuro-developmental

disorders, particularly autism spectrum disorder and language impairment [17,18]. The results of HLA analysis may vary in different populations due to their geographical distribution. Therefore, the results of similar studies undertaken in certain countries may not correspond to those of other research conducted in different locations with dissimilar populaces [19]. The present study aims to screen and investigate HLA class I and II in Indonesian OFC populations.

Material and Methods

Study Design and Sample

The present study is classified as case-control incorporating an accidental sampling approach. The participants were drawn from the Surabaya Cleft Lip and Palate Centre and comprised 15 OFC patients (ranging from 5-16 years old) and non-OFC individuals as the control group. The members of the latter group were matched by age to those of the OFC group without visible orofacial clefts, a history of cleft lip and palate (CLP) surgical reconstruction, or a family history of clefts. Seven mL of peripheral blood were obtained from all participants using EDTA, centrifuged to obtain plasma and subsequently frozen at -80oC to enable further analysis.

Laboratory Analysis

Enzyme-Linked Immunosorbent Assays (ELISAs) were performed on HLA-C and HLA-DR using specific antibodies according to the manufacturer's instructions. Human MHCC/HLA-C ELISA Kit (Catalog Number: E-EL-H2331) (Elabscience Biotechnology Co., Ltd, Houston, TX, USA) and Human HLA-DR ELISA Kit (ab223593) (Abcam, Cambridge, UK) were applied in the present study. All reagents and samples were prepared at room temperature prior to use. 1X Cell Extraction Buffer, 1X Wash Buffer PT, Antibody Cocktail were formulated according to the manufacturer's instructions. The standard working solution was prepared by performing serial dilution of stock standard for eight times, with standard #8 contains no protein and is the blank control. 50 µL of standard and samples were added to appropriate wells. Each sample was assayed with three replicates for statistical reasons. 50 µL was added to all wells. Incubation was performed for one hour at room temperature. Each wells were aspirated and washed three times using 350 µL 1X Wash Buffer, followed by adding 100 µL TMB substrate to each well and incubated for 10 minutes. 100 µL Stop solution was added to terminate the reaction. The optical density was measured in less than 30 minutes after the reaction had been terminated using an ELISA reader at wavelengths of 450 nm.

Data Analysis

The results were statistically analyzed using an independent t-test to compare each variable with the normal value from the control group. A p-value lower than 0.05 was considered significant. Data were analyzed to compare the CLP group and control group using IBM SPSS Statistics Software (IBM Corp., Armonk, NY, USA). A Kolmogorov-Smirnov test was performed to analyze normality.

Ethical Aspects

This research protocol was approved by the Ethics Committee of the Faculty of Dental Medicine, Airlangga University (094/HRECC.FODM/III/2019). The objectives, risks, side-effects, and supplementary information of this research were all fully explained to the children and their parents or legal guardians, with written consent being obtained from all participants.

Results

Orofacial cleft subjects were recruited in accordance with the LAHSAL system and their distribution is presented in Table 1.

Table 1. Distribution of study participants.

Sex	Bilateral Cleft Lip and Palate	Unilateral Cleft Lip and Palate	Total
	N	N	
Male	4	1	5
Female	5	5	10
Total	9	6	15

Of the bilateral cleft lip and palate patients, five possessed incomplete clefts diagnosed as LAHSAL, HSAL, LAHS—1, LAHSh– and La–-aL. Those patients with unilateral cleft lip and palate consisted of one male and five females diagnosed with SHAL and AL. None presented a cleft lip-only (CLO) or cleft palate-only (CPO) phenotype. The results of the ELISA relating to HLA-C and HLA-DR are shown in Table 2.

Table 2. Distribution of HLA-C and HLA-DR measurement.

Variables	Mean	Median	Minimum Value	Maximum Value	SD
HLA-C					
CLP	1.91	1.86	1.54	2.37	0.23
Control	2.29	2.33	1.84	2.59	0.26
HLA-DR					
CLP	0.06	0.06	0.03	0.08	0.01
Control	0.13	0.12	0.08	0.19	0.04

The values of HLA-C and HLA-DR in the male and female participants were similar with a difference of 0.07. The measurement of soluble HLA-C in the present study was $p<0.01$. The data revealed significant differences in both soluble HLA-C and HLA-DR levels between the CLP group and the control group.

Discussion

The distribution of HLA may vary between ethnic and geographic populations, while the results of HLA-related studies remain inconsistent between populations [19]. MHC, which is located in chromosome 6p21, consists of nearly 150 genes that encode HLA proteins. Due to their proximity, the incidence rate of the inheriting of these genes varies across populations worldwide. Cell surface receptors were encoded by MHC genes and categorized according to their ability to process and present antigens to T cells. HLA class I were expressed in all nucleated cells and presented antigen fragments intracellularly to CD8+ T cells. A cytokine-mediated immune response was subsequently initiated. Unlike MHC class I cells, their MHC class II counterparts were only found in antigen presenting cells (APC) such as B cells, macrophages and microglia. Its main role is to present exogenous material within the cells via endocytic vesicles to CD4+ T cells [20].

Several attempts to analyze the association of CLP with HLA have been conducted. HLA locus is associated with the pathogenesis of CLP in mice placed on a prolonged course of cortisone treatment [21]. Long-term maternal glucocorticoid consumption has been associated with a higher risk of the occurrence of CLP [22]. To the best of the authors' knowledge, a similar investigation analyzing parental corticosteroid consumption with the occurrence of CLP and HLA expression in humans has not been reported to date. Increased expression of HLA-DR has also been discovered in infants with CLP compared to healthy infants [23].

The mechanism involved in the HLA complex, possibly affecting the pathogenesis of CLP, has yet to be firmly established, even though a significant number of studies have consistently reported the association. A higher frequency of HLA-A, HLA-B and HLA-DR among cleft patients compared to that observed in a healthy population has also been reported. The frequency of HLA-A has also increased among the Caucasian and Mexican-American populations, although data relating to the Asian population has yet to be collated. HLA studies on Japanese populations have revealed that the frequency of HLA-C was significantly higher compared to that of a non-cleft phenotype. Despite this positive correlation, several studies have failed to analyze the prevalence of HLA complex among CLP patients [24]. In this study, the HLA-C and HLA-DR were examined due to geographical variations and represented both classes of HLA: HLA class I consisting of HLA-A, HLA-B and HLA-C, and HLA-DR representing HLA class II. The data produced in the course of the present study indicated that the levels of HLA-C and HLA-DR were significantly increased in CLP patients.

In addition to genetic factors, environmental ones also contribute to the etiology of CLP. Gene mutation, whether involving single genes or polygenic in nature, may constitute the predominant hereditary pattern. Every mutation creates a harmful effect that induces certain pathological conditions. It is widely known that each individual possesses genetic susceptibility to various conditions, including oral cleft. The phenotype develops if the stimulus is above the threshold level, whereas no cleft phenotype will occur if it is below that level. The combined individual susceptibility of parents will be inherited by their offspring. Thus, the offspring are prone to cleft phenotype. The probability of oral cleft is amplified when genetic susceptibility is triggered by environmental factors that affect the mother prior to or during pregnancy [24].

The findings of the present study are particularly useful in profiling the levels of soluble protein HLA-C and HLA-DR in CLP patients, which constitute essential preliminary data for further research leading to a deeper understanding of HLA and CLP. The major limitation of this study was the lack of patient and parent participation in the assessment of maternal nutrition levels due to geographical and social factors. CLP is considered both a burden and an embarrassment to the affected families in most developing countries, including Indonesia [25,26]. Delivering treatment and conducting research related to CLP remains most challenging in rural areas where healthcare remains inaccessible and members of the local population are largely unaware of their oral health.

Despite these obstacles, the present study provides preliminary data for future research, particularly that intended to reveal the roles of HLA in non-infectious and non-autoimmune medical conditions. The studies relating to CLP in Indonesia have focused mainly on the clinical and social aspects. To the best of the authors' knowledge, no previous reports relating to HLA and CLP patients in Indonesia exist. It is, therefore, their intention to provide information regarding the important roles of HLA in a variety of biological processes and its potential utility as a biomarker for certain medical conditions.

Conclusion

The association of HLA with the pathogenesis of CLP remains controversial and represents an opportunity for further exploration. Nevertheless, the data reported in this study reveals that there were increased levels of HLA-C and HLA-DR in the CLP group, suggesting that the variables examined may affect specific pathways involved in the pathogenesis of CLP and predispose certain individuals to the oral cleft. More exhaustive investigations to ensure correlation would support early diagnosis and the planning of therapy, as well as providing educational information for the family involved.

Authors' Contributions

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All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

None.

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References

[1] Pamplona MC, Ysunza PA. Language proficiency in children with cleft palate. Int Arch Commun Disord 2018; 1(1):003. https://doi.org/10.23937/iacod-2018/1810003

[2] Mossey P, Little J. Addressing the challenges of cleft lip and palate research in India. Indian J Plast Surg 2009; 42(Suppl):S9–S18. https://doi.org/10.4103/0970-0358.57182

[3] Burg ML, Chai Y, Yao CA, Magee W III, Figueiredo JC. Epidemiology, etiology, and treatment of isolated cleft palate. Front Physiol 2016; 7:67. https://doi.org/10.3389/fphys.2016.00067

[4] Shkoukani MA, Chen M, Vong A. Cleft lip – a comprehensive review. Front Pediatr 2013; 1:53. https://doi.org/10.3389/fped.2013.00053

[5] Goldberg AC, Rizzo LV. MHC structure and function – antigen presentation. Part 1. Einstein 2015; 13(1):153-6. https://doi.org/10.1590/S1679-45082015RB3122

[6] Hosomichi K, Shiina T, Tajima A, Inoue I. The impact of next-generation sequencing technologies on HLA research. J Hum Genet 2015; 60(11):665-73. https://doi.org/10.1038/jhg.2015.102

[7] Mosaad YM. Clinical role of human leukocyte antigen in health and disease. Scand J Immunol 2015; 82(4):283-306. https://doi.org/10.1111/sji.12329

[8] Alves C, Vieira N, Meyer I, Alves CO, Toralles MBP, Oliveira MFSP. Human histocompatibility antigens and dermatology: from research to clinical practice. An Bras Dermatol 2006; 81(1):65-73. https://doi.org/10.1590/S0365-05962006000100009

[9] Bodis G, Toth V, Schwartig A. Role of human leukocyte antigens (HLA) in autoimmune diseases. Rheumatol Ther 2018; 5(1):5-20. https://doi.org/10.1007/s40744-018-0100-z.

[10] Insel R, Duta S, Hedrick J. Type 1 diabetes: disease stratification. Biomed Hub 2017; 2(Suppl. 1):481131. https://doi.org/10.1159/000481131

[11] Dendrou CA, Petersen J, Rossjohn J, Fugger L. HLA variation and disease. Nat Rev Immunol 2018; 18(5):325-39 https://doi.org/10.1038/nri.2017.143

[12] Akassou A, Bakri Y. Does HLA-B27 status influence ankylosing spondylitis phenotype?. Clin Med Insights Arthritis Musculoskelet Disord 2018; 11:1179544117751627. https://doi.org/10.1177/1179544117751627

[13] Hollenbach JA, Norman PJ, Creary LE, Damotte V, Montero-Martin G, Caillier S, et al. A specific amino acid motif of HLA-DRB1 mediates risk and interacts with smoking history in Parkinson's disease. PNAS 2019; 116(15):7419-24. https://doi.org/10.1073/pnas.1821778116

[14] Valenzuela-Ponce H, Alva-Hernández, Garrido-Rodríguez D, Soto-Nava M, García-Téllez T, Escamilla Gómez T, et al. Novel HLA class I associations with HIV-1 control in a unique genetically admixed population. Sci Rep 2018; 8(1):6111. https://doi.org/10.1038/s41598-018-23849-7

[15] Califf RM. Biomarker definitions and their applications: minireview. Experim Biol Med 2018.; 243(3):213-21. https://doi.org/10.1177/1535370217750088

[16] Matzaraki V, Kumar V, Wijmenga C, Zhernakova A. The MHC locus and genetic susceptibility to autoimmune and infectious diseases. Genome Biol 2017; 18(1):76. https://doi.org/10.1186/s13059-017-1207-1

[17] Nudel R, Simpson NH, Baird G, O'Hare A, Conti-Ramsden G, Bolton PF, et al. Association of HLA alleles with specific language impairment. J Neurodev Disord 2014; 6(1):1. https://doi.org/10.1186/1866-1955-6-1

[18] Bennabi M, Gaman A, Delorme R, Boukouaci W, Manier C, Scheir I, et al. HLA-class II haplotypes and autism spectrum disorders. Sci Rep 2018; 8(1):7639. https://doi.org/10.1038/s41598-018-25974-9

[19] Buhler S, Nunes JM, Sanchez-Mazas A. HLA Class I molecular variation and peptide-binding properties suggest a model of joint divergent asymmetric selection. Immunogenetics 2016; 68(6-7):401-16. https://doi.org/10.1007/s00251-016-0918-x

[20] Wieczorek M, Abualrous ET, Sticht J, Álvaro-Benito M, Stolzenberg S, Noé F. Major histocompatibility complex (MHC) class I and MHC class II proteins: conformational plasticity in antigen presentation. Front Immunol 2017; 8:292. https://doi.org/10.3389/fimmu.2017.00292

[21] Marazita ML. The evolution of human genetic studies of cleft lip and cleft palate. Annu Rev Genomics Hum Genet 2012; 13:263-83. https://doi.org/10.1146/annurev-genom-090711-163729

[22] Bandoli G, Palmsten K, Smith CJF, Chambers CD. A review of systemic corticosteroid use in pregnancy and the risk of select pregnancy and birth outcomes. Rheum Dis Clin North Am 2017; 43(3):489-502. https://doi.org/10.1016/j.rdc.2017.04.013

[23] Inoyatov A, Abdurahmanov M, Sharopov S, Azimov M. The condition of immune system of infants with congenital cleft lip and palate. Med Health Sci J 2012; 10:23-9. https://doi.org/10.15208/mhsj.2012.5

[24] Rajendran R, Shaikh SF, Anil S. Tracing disease gene(s) in non-syndromic clefts of orofacial region: HLA haplotypic linkage by analyzing the microsatellite markers: MIB, C1_2_5, C1_4_1, and C1_2_A. Indian J Hum Gen 2011; 17(3):188-93. https://doi.org/10.4103/0971-6866.92101

[25] Adeyemo WL, James O, Butali A. Cleft lip and palate: parental experiences of stigma, discrimination, and social/structural inequalities. Ann Maxillofac Surg 2016; 6(2):195-203. https://doi.org/10.4103/2231-0746.200336

[26] Shirol SS. Sociocultural beliefs and perceptions about cleft lip-palate and their implications in the management, outcome, and rehabilitation. J Cleft Lip Palate Craniofac Anomal 2018; 5:4-5. https://doi.org/10.4103/jclpca.jclpca_41_17

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