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OriginalResearchArticle

Association of ABO blood group and Rh factor in cleft lip and palate patients

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ABSTRACT

Background: One of the most common congenital malformations, with widespread racial and regional variation is an orofacial cleft. The occurrence is attributed to an array of environmental and genetic factors. Blood grouping and Rh factor are genetically determined. Any possible association of clefts with them helps in planning interventional services.
Methods: A case control observational study was conducted on 111 samples who were cases presenting with oral clefts in Super speciality hospitals and other 111 samples who came to hospital for their treatment other than for cleft lip or cleft palate, were controls in the study. Cases were evaluated for various phenotypes of clefts. Blood samples of each case and control was collected to elaborate on blood group genotype and Rh typing. SPSS 22.0 version was employed for statistical analysis**.**
Results: The most common blood group noted in cases as well in controls respectively was type 'B' in 31.5% and 43.2%, while blood group 'AB' was noted the lowest in both cases (14.5%) and controls (7.3%).Rh positive was noted 94.6% in both cases and control population. Clefts were observed more in male population than female counterparts.Cases of cleft lip and palate (CLP) was noted the highest, in 61 (55%) of cases, followed by defects in lip, palate and lastly in soft palate.
Conclusions: Though not associated to the biological characteristics of cleft lip and palate in the current study, the functional importance of ABO blood group distribution may be the subject of future research. Identification of any associative traits for clefts assesses individuals with risk so as to help eliminate the chance of occurrence and early identification for better prognosis.

Keywords: Blood groups, Genetics, Oral clefts, Rh Factor, Unilateral

INTRODUCTION

Cleft lip and cleft palate remain the common morphological defect afflicting the oro-facial structures in mankind, and remains as a topic of considerable interest with regards to aetiology and pathogenesis of these defects. Functional and esthetical implications have led to extensive research in terms of aetiology and intervention strategies.[1]Literature shows the incidence rate of oral clefts to be 1.42 in 1000.[2] Etiopathogenesis concept of

recent evolve suggests that clefts are a heterogenous category of craniofacial defects due to interplay of environmental and genetic factors.[3]Among the many elements investigated in this relationship are: maternal usage of tobacco, alcohol or substance usage, folic acid deficiency in the diet, maternal obesity and gestational diabetes, exposure to teratogenic drugs and environmental contaminants. There are several epigenetic variables that are linked to clefts such as increased parental age, consanguinity, and family history. Other determinants such as maternal stress and assisted reproduction are also investigated. Forecasting Cleft lip and palate might be considered a significant step forward toward prevention and facilitates early access genetic services. The geneticist can determine the extent to which genetic factors are involved in the aetiology of cleft lip and cleft palate in a given problem based on a good family history and provide the parents with a prognosis for recurrence of the condition in future siblings as well as the potential for affected offspring arising from already affected children through genetic counselling.[4]ABO blood grouping and Rh factor antigens are genetically determined, playing an important role in comprehending inheritance patterns, population genetic studies, and disease susceptibility, including Oral facial clefts. So, blood groups could act as a cue to further analyse individual gestational risk factors which could aid in better service planning, especially among disadvantaged and vulnerable communities. Hence this study was undertaken to determine association of oral clefts with ABO and Rh factor in North Indian population.

Aims and objectives

The primary objective of the study was to find out if any association of blood group type and Rh factor with Cleft Lip and Palate (type of cleft and side of cleft) in case group. The secondary objective was to compare the blood group and Rh factor between controls and cases.

METHODS

A case control observational hospital-based study was conducted to assess for any possible association of Blood grouping and Rh typing among cleft lip and cleft palate cases and controls. The study was conducted for a duration of 5 months from January 2022 to May 2022. Random sampling was done to collect samples. Consent of all participants were obtained from both cases and controls, butin case of child patient, consent was obtained from guardian/ parent or caretaker, who accompanied the child.

Screening of cases and controls

Patients with cleft lip and palate attending Outdoor patient department of Sushrut institute of plastic surgery Lucknow and Plastic surgery Department of King George Medical college Lucknow were recruited by two plastic surgeons to rule out any syndromic case of Cleft lip and palate and only all non-syndromic CLP patients formed the case group. 120 cases of CLP were recruited but only 111 participated in the study. Same number of controls were recruited for the study. Patients coming to the hospital for their treatment other than for CLP treatment formed the control group. All the patients who were free from any CLP defects formed the control group. A written proforma was filled out for each case and control eliciting demographic details such as age, gender, cleft details, family income, marital status (consanguineous/ non-consanguineous), education, occupation, parent's history. The severity and type of cleft was recorded based on Kernahan's classification.[5]

Blood sample collection

Blood collection was done under strict aseptic conditions. 3 ml of venous blood was collected from median cubital vein through venepuncture via disposable syringe. This was then transferred to vacutainer tube containing K3 ethylene diamine tetraacetic acid. Collected blood was immediately stored at 4 degrees centigrade to avoid any hemolysis of RBCs for a maximum of 40 days. Within the period of 40 days from sample collection we had checked ABO blood group and Rh Factor in each case or control group. ABO blood grouping and Rh typing was based on the principle of forward typing of blood grouping using Eryscreen kit.[6] These kits are commercially established and validated. They are fast and reliable and give results in 5 minutes. ensure accuracy between readings, both Rh typing and Blood group testing was done in the department of hematology Sanjay Gandhi post graduate Institute Lucknow. Elaborate details on clefts were recorded. Clefts were categorised separately as cleft lip (CL), cleft palate(CP), combination of both cleft lip and palate (CLP) and clefts of the soft palate. Location of these clefts were also recorded based on unilateral, bilateral or midline existence. Any other congenital anomaly present in any case, was not included in our sample population.

Statistical analysis

The continuous data were summarised in mean±SE (standard error of the mean) whereas discrete (categorical) in number (N) and percentage (%). Continuous two independent groups were compared by Student's t test whereas categorical groups were compared by Chi-square (χ^2) test. A two-tailed (α=2) $p<0.05$ was considered statistically significant. Analysis was performed on SPSS software (windows version 22.0).

RESULTS

The present study assesses blood group type and Rh factor association with cleft lip and palate patients. Total 222, 111 normal patients without cleft lip and palate and 111 with cleft lip and palate patients were recruited and

served as controls and cases respectively. The outcome measures of the study were blood group type, Rh factor, type of cleft and side of cleft. The blood group type and Rh factor were assessed in both controls and cases whereas type of cleft and side of cleft were assessed in cases only.

Basic characteristics

The basic characteristics of two groups (controls and cases) at presentation (enrolment) were summarised in (Table 1).

Table 1: Basic characteristics of two groups (n=111).

Variable	Controls N (%)	Cases N (%)	t/χ^2 value	P value
Age (yrs); (mean±SD)	38.80±2.06	5.15±0.67	15.52	< 0.001
Sex				
Female	50 (45.0)	34 (30.6)	4.90	0.027
Male	61 (55.0)	77 (69.4)		
Blood group				
A	27 (24.3)	31 (27.9)	5.00	0.172
AB	8 (7.3)	16 (14.5)		
B	48 (43.2)	35 (31.5)		
O	28 (25.2)	29 (26.1)		
Rh factor				
Negative	6 (5.4)	6 (5.4)	0.00	1.000
Positive	105 (94.6)	105 (94.6)		
Type of cleft				
CL	-	33 (29.7)	-	-
CLP	-	61 (55.0)		
CP	-	14 (12.6)		
Soft palate	-	3 (2.7)		
Side of cleft				
Bilateral	-	14 (12.6)	-	-
Left	-	53 (47.7)		
Middle	-	17 (15.3)		
Right	-	27 (24.3)		

The age of two groups were summarised in Mean±SE and compared by t test (t value) whereas blood group and Rh factor were summarised in number (N) and percentage (%) and compared by chi-square (χ^2) test (χ^2 value). NA: not applicable.

Table 2: Correlation between blood group and Rh factor in controls (n=111).

Rh factor	Blood group				χ^2 value	P value
	A(N=27) Frequency (%)	AB (N=8) Frequency (%)	B (N=48) Frequency (%)	O (N=28) Frequency (%)		
Negative	11 (40.7)	5 (62.5)	20 (41.7)	14 (50.0)	1.69	0.640
Positive	16 (59.3)	3 (37.5)	28 (58.3)	14 (50.0)		

Correlation between blood group type and Rh factor type in controls was summarised number (N) and percentage (%) and compared by Chi-square (χ^2) test (χ^2 value).

The mean age and frequency (%) of sex proportions (M/F) differ significantly ($p<0.05$ or $p<0.001$) between the two groups. In controls, the frequency of blood group type A, AB, B and O were 24.3%, 7.3%, 43.2% and 25.2% respectively whereas in cases it was 27.9%, 14.5%, 31.5% and 26.1% respectively. In both groups, the frequency of blood group type B was the highest with 11.7% higher being in controls (43.2%) as compared to cases (31.5%). In contrast, in both groups, the frequency of blood group type AB was the minimum but 7.2% higher in cases (14.5%) as compared to controls (7.3%). However, the difference in frequency of blood group types did not ($p>0.05$) differ between the two groups i.e., found to be statistically the same. Further, in controls, 6 (5.4%) subjects had Rh factor negative and 105 (94.6%) had Rh factor positive. The cases showed exactly similar frequency of negative and positive Rh factor thus the difference being also insignificant ($p>0.05$) between the two groups. Furthermore, in cases, the frequency of type of cleft CL, CLP, CP and soft palate were 29.7%, 55.0%, 12.6% and 2.7% respectively with highest being of CLP (55.0%) and soft palate the least (2.7%).Moreover, in cases, the frequency of side of cleft bilateral, left, middle and right were 12.6%, 47.7%, 15.3% and 24.3% respectively with highest being at left side (47.7%) and bilateral the least (12.6%).

Correlations

Controls: The correlation (association) between blood group type and Rh factor type in controls is summarised

in (Table 2). In controls, the frequency of Rh factor negative was highest in blood group AB (62.5%) whereas positive was highest in blood group A (59.3%). The χ^2 test showed insignificant (p>0.05) association between blood group type and Rh factor type in controls.

Cases: Blood group; The correlation (association) of Rh factor type, type of cleft and side of cleft with blood group type in cases is summarised in (Table 3).

Table 3: Correlation of Rh factor, type of cleft and side of cleft with blood group in cases (n=111).

Variable	Blood group				χ^2 value	P value
	A (N=31) Frequency (%)	AB (N=16) Frequency (%)	B (N=35) Frequency (%)	O (N=29) Frequency (%)		
Rh factor						
Negative	2 (6.5)	1 (6.3)	2 (5.7)	1 (3.4)	0.31	0.958
Positive	29 (93.5)	15 (93.8)	33 (94.3)	28 (96.6)		
Type of cleft						
CL	9 (29.0)	4 (25.0)	10 (28.6)	10 (34.5)	2.90	0.968
CLP	18 (58.1)	8 (50.0)	20 (57.1)	15 (51.7)		
CP	3 (9.7)	3 (18.8)	4 (11.4)	4 (13.8)		
Soft palate	1 (3.2)	1 (6.3)	1 (2.9)	0 (0.0)		
Side of cleft						
Bilateral	4 (12.9)	2 (12.5)	4 (11.4)	4 (13.8)	2.39	0.984
Left	15 (48.4)	6 (37.5)	19 (54.3)	13 (44.8)		
Middle	4 (12.9)	4 (25.0)	5 (14.3)	4 (13.8)		
Right	8 (25.8)	4 (25.0)	7 (20.0)	8 (27.6)		

Correlation of Rh factor type, type of cleft and side of cleft with blood group in cases was summarised number (n) and percentage (%) and compared by Chi-square (χ^2) test (χ^2 value).

Table 4: Correlation of type of cleft and side of cleft with Rh factor in cases (n=111).

Variable	Rh factor		χ^2 value	P value
	Negative (N=6) Frequency (%)	Positive (N=105) Frequency (%)		
Type of cleft				
CL	2 (33.3)	31 (29.5)	23.39	< 0.001
CLP	2 (33.3)	59 (56.2)		
CP	0 (0.0)	14 (13.3)		
Soft palate	2 (33.3)	1 (1.0)		
Side of cleft				
Bilateral	1 (16.7)	13 (12.4)	1.86	0.603
Left	2 (33.3)	51 (48.6)		
Middle	2 (33.3)	15 (14.3)		
Right	1 (16.7)	26 (24.8)		

Correlation of type of cleft and side of cleft with Rh factor in cases was summarised number (n) and percentage (%) and compared by Chi-square (χ^2) test (χ^2 value).

In cases, Rh factor, type of cleft and side of cleft did not (p>0.05) correlate well with blood group thus suggesting that Rh factor, type of cleft and side of cleft not found to be associated with blood group in cleft lip and plate patients. Rh factor: The correlation (association) of type of cleft and side of cleft with Rh factor in cases is summarised in (Table 4). In cases, the type of cleft showed significant (p=0.001) association with Rh factor; however, side of cleft did not (p>0.05) show any association. The type of cleft was thus found to be associated significantly with Rh factor in cleft lip and plate patients but not the side of cleft.

DISCUSSION

Discovery of ABO blood group systems by Karl Landsteiner marked a significant milestone in science. Research conducted on blood groups disclosed several parameters in genetic aspects facilitating an understanding of disease pathogenesis, thus reducing prevalence and severity of the disease condition.[7,8]The scientific community is devoting considerable attention to the relation between ABO and Rh blood group characteristics and various illnesses. Greer et aldiscovered that those with the O phenotype have a higher risk of developing pancreatic cancer. When

infected with Vibrio cholerae, people with the O phenotype were more likely to acquire symptomatic illness, according to Harris et al, Tamega et al discovered that the A phenotype predominates in people with chronic discoid lupus erythematosus, making prognosis in diffuse clinical manifestations of the illness easier.[7-11] Sharma et al found that those with the B phenotype had a greater frequency of malocclusions in the population of Jaipur, India.[12]Maor et al said that there is increased risk of orofacial clefts in children born with assisted reproduction.[13]Hence a study to associate the presence of oral clefts to blood group phenotyping and Rh is imperative in enhancing our understanding of these craniofacial malformities so as to embrace necessary preventive measures. While searching for the relevant literature very few studies had been done to see the association between blood groups and Cleft lip and palate patients. So, this study could through some light on the association. This study is one of its kind to study blood group association in CLP patients mainly in Northern India. In literature search no other study exists on North Indian population showing association with blood groups and CLP. When comparing for the phenotypic distribution of clefts in our study, Cleft lip and palate was of predominance noted in 55 % of case population. It is dissimilar to the study of Jamilian wherein 42.2% of their cases presented with Clefts in palate.[14] Our study demonstrated a highest frequency of blood group 'B' in cases with Orofacial clefts as well as in controls which represents the normal population of North India. These findings are supported by other study of Ling et al from China Medical University in Northeast China.[15] They concluded that B blood group is more frequent in cleft lip palate patients but it is not statistically significant and occurrence of CLP is not associated to ABO blood groups. Our study also showed that control sample (Normal population) had a highest percentage of 'B' blood group. This finding is in concordance to the study of Agarwal et al which says that normal blood group in North Indians is 'B+'. In our study when we noticed within blood group A, it is more in case group as compared to control group.[16]

Mushriqet also saw the same trend in their study, they had studied 245 cleft children without taking any control group.[17] In our study we saw that there are 7.2% more 'AB' blood group CLP cases as compared to controls that is twice to the normal population. This finding is similar to that of Hussain et al who reported 'AB' blood grouping (25.8%) to be 5 times higher in CL/CP patients in comparison to their normal Iranian counterparts (4.9%).[4] However the study of Sivanand et al who studied Dravidian population of Chennai, reported a greater proportion of the blood group 'A' as compared to normal population which has higher percentage of "O' blood group and found it statistically significant.[18] In our study occurrence of CLP is not associated with ABO and Rh factor and it is not statistically significant. The findings of this study are in agreement with other studies.

Most of the unilateral cases of oral clefts in our study was noted in the left side. As per the proposed probability of Johnston et all and Carroll et al the right side of the head is better nourished as the vessels supplying them diversify at close proximity to the heart when compared to the contralateral side.[19,20] The literature review of Vyas et al further reinforced this fact, by stating that a ratio of 4:1 for unilateral clefts to bilateral types, with about 70% occurring on the left facial side.[21]Oral clefts need to be intervened early for to decrease secondary deformities with functional capacity restored to a maximum potential and for better prognosis. Also, cleft lip/cleft palate affected children because of lesser attractive aesthetics or disrupted speech are often the soft targets of teasing by their peers.

Significance and future prospects

The geneticist can assess from a good family history that which genetic factors are involved in occurrence of CLP. An understanding of the genetic predisposition of Oral clefts provides geneticist for determining the recurrence of these in future siblings and the probability of an offspring born with the condition in affected individuals. However, the findings of the study should not be taken independently rather be compared with candidate genes and other risk factors to increase the quality of evidence. A study could be planned with larger sample size to conclude the results and with the help of extracted DNA, Single nucleotide polymorphisms could be diagnosed for any occurrence of CLP.

Limitations

No effort was made to explore the ethnicity of the study population due to absence of any systematic information about mother's race. Further increase in mixed marriages makes racial tracing difficult. In this study pattern of inheritance of blood groups was not taken into account as it was not part of the study.

CONCLUSION

It was found that 'B' blood group frequency was the highest both in case as well as control groups as compared to other blood groups but it is more in control group by 14.5%. It is also seen that blood group 'A' is more in case groupas compared to control group. Blood group 'AB' is least both in case and control group but it is presented nearly twice more in case group as compared to control group. Major cases reported were on left side. Severe cases of clefts were seen mostly in males. Rh factor occurrence is same both in case and control groups. Further studies with larger sample sizes and varied ethnicity are recommended to explore this genetic analysis.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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