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THE EFFECT OF GENETIC AND ENVIRONMENTAL FACTORS ON THE DEVELOPMENT OF DENTITION –A STUDY ON TWINS

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ABSTRACT:

Aim: The aim of the study was to determine the extent of genetic influence and environmental contribution to dentofacial growth and development. **Materials and methods:** 15 pairs twins were selected and divided into monozygotic (8) and dizygotic(7) with the help of DNA finger printing analysis. Study models of the twins were taken. The parameters like overjet, overbite, U/L inter premolar width, U/L inter molar width, U/L arch length and palate depth were recorded on study cast. **Results:** Statistical analysis reveals that significant hereditary component for the parameters-overjet, overbite, U/L Inter premolar width, U/L Inter molar width and palate depth. No significant heritability was observed for U/L arch length. **Conclusion:** A significant heritability values were obtained for seven out of nine parameters studied. overjet, overbite, both upper and lower inter premolar width and inter molar width, palate depth were showing a significant genetic variability where as upper and lower arch length showed insignificant genetic influence, indicating environmental influences substantially.

KEYWORDS: Twins, Study cast, DNA finger printing, Heritability.

INTRODUCTION

Human growth is a complex process that begins with basic genetic inheritance but shaped by environmental factors. How much influence each of these factors has on shaping the development of human growth is not really known and is frequently debatable. In orthodontics, researchers stress the predominant role of heredity on the dentofacial complex. Kingsley (1879), Fox (1840), Brown (1841),Manry (1928), Bell (1929) etc. recognize the role of heredity in the development of number of dentofacial anomalies including malocclusion[1]. A number of recent studies also reiterate the existence of genetic influence on dentofacial morphology and etiology of malocclusion.

Lack of sufficient genetic data has been a limiting effect on progress towards the solution of some fundamental problems in anthropology and orthodontics. Though the form and proportions appear to be heritable, but the quantitative assessment of heritability is lacking. Consequently, attempts to establish morphological criteria for racial comparisons or orthodontic diagnostic purposes have been hampered.

The effect of heredity on dentofacial complex may be studied by several means such as animal studies, populations studies, family studies, twin studies, genetic typing.

They are two types of twins - monozygotic and dizygotic. Identical twins are the result of division of an egg after fertilization and have identical genotype and similarities in the most of the physical characteristics. Any differences in morphology attributed to the effect of environment.

Dizygotic twins are result of fertilization of two separate eggs and have different genotype. The differences in their physical characteristics are due to both genetic & environmental factors. Hence twin studies are proven effective in partitioning the effects on developing dentofacial complex in to genetic and environmental components (Coytic 1944, Lundstorm 1984, Hunter 1965, Nakata 1973)[2].

The purpose of the study is to evaluate the amount of genetic and environmental influences on dentofacial complex and dentition by using study models.

Material and Methods:

The subject of the present study consists of 15 pairs of twins with age ranging from 12-16 years. Facial photographs, blood sample (10ml), past H/o of habits and orthodontic treatment of the patients were recorded.

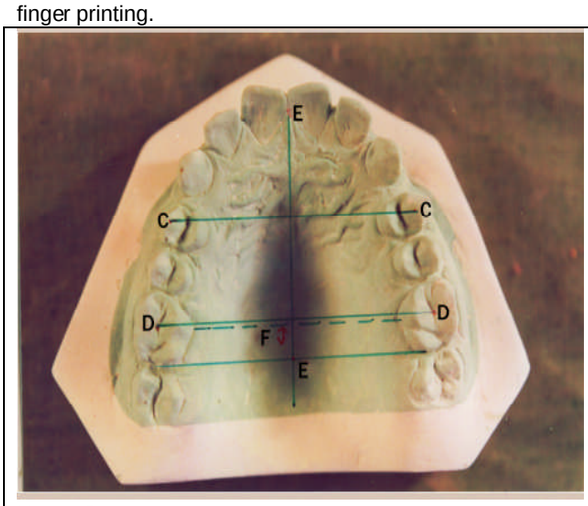
Study model :

Upper and lower study models were prepared with alginate impressions obtained from the twins. The following measurements were made on the study casts - over jet(OJ), over bite(OB), upper/lower Inter premolar

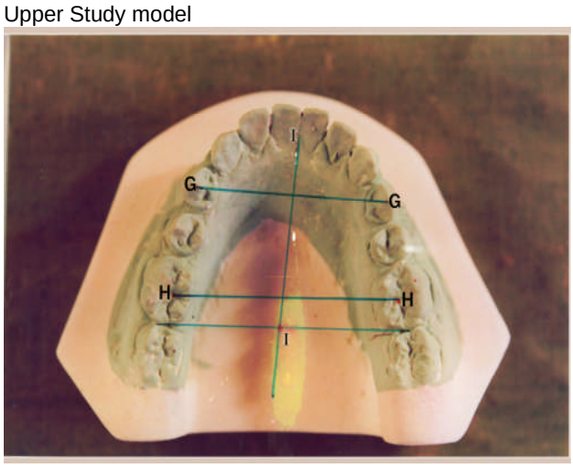
width(IPW- U/L), upper/lower Inter molar width(IMW-U/L), upper/lower arch length (AL-U/L), depth of the palate **(Fig.1).**

DNA Finger Printing:

Zygocity determination is important part of the study. It can be determined by external traits, dermatographics, serologic examination, genetic typing. Among all the procedures genetic typing is 100% accurate method to determine zygocity. Genetic typing is carried out by DNA finger printing.



Upper Study model



Lower study model

Fig.1. Variables measured on the study cast. C-C: Inter premolar width(Upper), D-D; Inter molar width(Upper), E-E: Arch length(Upper) , F-F: Palatal depth , G-G; Inter premolar width(Lower), H-H; Inter molar width(Lower), I-I: Arch length(lower)

Blood is best source for DNA. 10 ml blood was collected from each individual from all pairs of twins and subjected to DNA finger printing process. First step is isolation of DNA[3]. The purified DNA is divided in to fragments by using restriction enzymes. The fragment of DNA allowed to run through an electrophoresis gel[4] to separate then according to their size, when separation is completed, two strands of DNA are stripped apart in to single stand by a process of denaturation. Denaturated separated fragments are immobilized on the solid nitrocellulose or nylon membrane by a technique called vaccum blotting[5]. Once the fragments are immobilized on the solid support, hyper variable sequences are detected by radio actively labeled probe which hybridize with hydrogen bonding to complimentary sequences[6]. The probe hybridized fragments can be visualized by means of auto radiography, by keeping the X-ray film in direct contact with immobilized fragments and exposed areas appear as bands corresponding to hybridized fragments **(Fig.2).**

Analysis:

Heritability (H) is defined as the proportion of the phenotypic variance attributable to genetic source. To find out the intra pair difference, one number of the pair was taken as d_1 and the other as d_2. This was carried out for all parameters in both monozygotic and dizygotic twin pairs. From this mean variance is calculated. Using this mean variance F- ratio; Heritability (H); and standard error were calculated[7] by using the below mentioned formula[7].

$$V_{MZ} = \frac{d_1^2}{2N_1}$$

$$V_{DZ} = \frac{d_2^2}{2N_2}$$

$$F = \frac{V_{DZ}}{V_{MZ}}$$

$$\text{Heritability (H)} = \frac{V_{DZ} - V_{MZ}}{V_{DZ}}$$

$$V(H)\ 2. \frac{N^2_2}{N^2_1} \frac{(N_1 - 1)(N_1 + N_2 - 4)}{(N_2 - 3)(N_2 - 5)} . 1/F^2$$

$$SE(H) = \sqrt{V(H)}$$

V_{MZ} = Mean variance in monozygotic twin pairs.
V_{DZ} = Mean variance in dizygotic twin pairs.
N = Number of observations
H = Heritability
V(H) = Variance of Heritability
SE (H) = Standard error.

Results

The present study involves 15 pairs of twins, with age ranging from 12-16 years. After DNA finger printing analysis the twins were divided in to 8 monozygotic and 7 dizygotic twin pairs. Nine parameters on study cast were measured and recorded. The mean intra pair variance were calculated to each parameter in both mono and dizygotic twins **(Graph-1**) and F ratio, heritability **(Graph-2)** value (H), variance of Heritability V(H), standard error SE(H) and correlation coefficient calculated in both monozygotic and dizygotic twins.

The results showing significant heritability for the parameters like over Jet, over bite, inter premolar width(upper/lower), inter molar width(upper/lower), palatal depth. Whereas the parameters like arch length (upper/ lower) does not showing any significant heritability. The correlation coefficient of monozygotic twins shows the Inter premolar width in both upper and lower arches are positively correlated with inter molar width and arch length of both the arches**(Table-V).** Similarly maxillary arch length is positively correlated with mandibular arch length. The correlation coefficient of dizygotic twins shows positively correlation between maxillary inter premolar and maxillary inter molar width **(Table-VI).**

Discussion:

It is a proven reality that the genes play a definite key role in human growth and development. It is difficult to establish the genetic back ground of physical characteristics in human beings, because of the number of offspring in a family is small and span of generation is comparatively long and beyond the control of the investigator.

Starting with Galton, twins are being used in the investigation of Human genetics and related problem. Twin studies do not provide information regarding its mode of transmission. But do provide good information regarding genetic and environmental problems.

Determination of zygocity is very important in twin studies. This study was used the more accurate method i.e genetic typing (DNA finger printing).

Monozygotic twins are genetically similar and will have identical physical characteristics. Any differences observed are attributed to the environmental influences. Dizygotic twins are genetically dissimilar. The differences in physical characters are due to both genetic and environmental factors.

Overjet and Overbite: Overjet shows a mean intra pair variance of 0.2109 in monozygotic (**Table I**) and 3.2410 in dizygotic twin pairs **(Table II).** F ratio 15.3650**(Table III),** H 0.9349 (Table IV), SE (H) 0.1249(P< 0.001). Overbite shows a mean intra pair variance of 0.2518 in monozygotic **(Table I)** and 1.47 in dizygotic twin pairs **(Table II)**. F ratio 5.8362**(Table. III),** H 0.8286**(Table IV),** SE(H) 0.3288 (P<0.05). The values indicate high significance of genetic influence on overjet and overbite. The findings are not in agreement with Robert S Curracccini ,[8] Willian K Lobb,[2] Krishan Sharma,[9] A. Lundstram,[10] who noted less significant genetic variability and is in line with Salzmann, [11] who noted that the overjet and overbite are subjected considerably by genetic influence.

INTER PREMOLAR WIDTH (UPPER & LOWER): IPW(U) shows a mean intra pair variation of 0.3294 in monozygotic **(Table I)** and 4.8135 in dizygotic twin pairs(Table II). F ratio 14.6142(**Table III),** H 0.9315, SE (H) 0.1315(P<0.001)**(Table IV).** IPW(L) shows a mean intra pair variation of 0.2831 in monozygotic(Table I) and 3.3357 in dizygotic twin pairs(Table II). F ratio 11.7817, H 0.9152, SE (H) 0.16292. (P<0.001). The above values shows a highly significant genetic predominance on inter premolar widths of both upper and lower arches.

INTER MOLAR WIDTH (UPPER & LOWER): Inter molar width (upper) shows a mean intra pair variance 0.1656 in monozygotic(Table I) and 2.7042 in dizygotic twin pairs(Table II). F ratio 16.3277**(Table III),** H 0.9387(Table IV), SE (H) 0.1176 (P<0.001). Inter molar width (lower) shows a mean variance of 0.2106 in monozygotic**(Table I)** and 2.0757 in dizygotic twins(Table II). F ratio 9.8550(Table III), H 0.8985**(Table IV),** SE (H) 0.1947 (P<0.001). The variation for inter molar width in both the upper and lower arch showing a greater genetic determination. This finding conform the observations of Armindo Requelmi,[12] Krishanan Sharma, [9] Salzmann, [11] who noted a positive genetic influence on IPW and IMW.

ARCH LENGTH (UPPER AND LOWER):Arch length (upper) shows a mean intra pair variation of 0.6478 in monozygotic **(Table I)**and 1.7492 in dizygotic twin pairs(Table II). F ratio 2.7002**(Table III).** H 0.6296 **(Table IV)**, SE (H) 0.7108 (P>0.05). Arch length (lower) shows a mean intra pair variance of 0.7695 in monozygotic **(Table I)**and 2.0392 in dizygotic twin pairs. F ratio of 2.6500. H 0.6226, SE(H) 0.7243 (P>0.05) showing little genetic influence. Burton. L. Shapiro[13] also noted a similar observation.

PALATE DEPTH: Palate depth shows a mean intra pair variance of 0.1718 in monozygotic, 5.4871 in dizygotic twin pairs. F ratio 31.9251. H. 0.9686. SE(H) 0.0601 (P<0.001). The values indicate a highly significant genetic variability. The findings of Armindo Riquelme,[12] Krishan Sharma,[9] Salzmann,[11] Burton L Shapiro[13] also show a similar statistical significance.

Correlation co-efficient of monozygotic twins shows significant positive correlation between Inter premolar width in both upper and lower arches and inter molar width

Table.1. Mean Intrapair Differences On Study Cast Dimensions Of MonozygoticTwin Pairs

S.No	Parameters	Number of Mz Twin pairs	Mean Dz Intrapair Difference (mm)
1.	OVER JET	8	0.2109
2.	OVER BITE	8	0.2518
3.	IPW-U	8	0.3294
4.	IPW-L	8	0.2831
5.	IMW-U	8	0.1656
6.	IMW-L	8	0.2106
7.	AL-U	8	0.6478
8.	AL-L	8	0.7695
9.	PALATAL DEPTH	8	0.1718

Table.2.Mean Intrapair Differences On Study Cast Dimensions Of Dizygotic Twin Pairs

S.No	Parameters	Number of Dz Twin pairs	Mean Dz Intrapair Difference (mm)
1	OVER JET	7	3.241
2	OVER BITE	7	1.47
3	IPW-U	7	4.8135
4	IPW-L	7	3.3357
5	IMW-U	7	2.7402
6	IMW-L	7	2.0757
7	AL- U	7	1.7492
8	AL- L	7	2.0392
9	PALATAL DEPTH	7	5.4871

Table - 3 F- Ratio Of Study Cast Variables

S.No	Parameters	Number of Dz Twin pairs	F-Ratio	Level of Significance
1.	OVER JET	15	15.3650	< 0.01*
2.	OVER BITE	15	5.8362	< 0.05*
3.	IPW-U	15	14.6142	< 0.01*
4.	IPW-L	15	11.7817	< 0.01*
5.	IMW-U	15	16.3277	< 0.01*
6.	IMW-L	15	9.8550	< 0.01*
7.	AL- U	15	2.7002	> 0.05
8.	AL- L	15	2.6500	> 0.05
9.	PALATAL DEPTH	15	31.9251	< 0.01*

***--Significant**

Table - 4 Heritability Coefficients Of Study Cast Variables

S.No	Parameters	Number of Twin pairs	H	SE(H)	Level of Significanc
1.	OVER JET	15	0.9349	0.1249	< 0.001*
2.	OVER BITE	15	0.8286	0.3288	< 0.05 *
3.	IPW-U	15	0.9315	0.1315	< 0.001*
4.	IPW-L	15	0.9152	0.1692	< 0.001*
5.	IMW-U	15	0.9387	0.1176	< 0.001*
6.	IMW-L	15	0.8985	0.1947	< 0.001 *
7.	AL- U	15	0. 6296	0.7108	> 0.05
8.	AL- L	15	0.6226	0.7243	> 0.05
9.	PALATAL DEPTH	15	0.9686	0.0601	< 0.001*

***--Significant**

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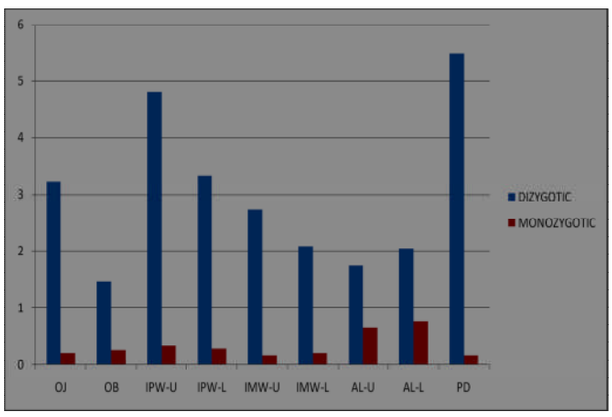
Table-5. Correlation Coefficients Of Monozygotic Variables

	1	2	3	4	5	6	7	8	9
1	1	0.234	-0.34	-0.15	-0.32	-0.38	0.095	0.253	0.58
2	0.234	1	-0.12	-0.01	0.012	-0.04	-0.10	-0.03	065
3	-0.34	-0.12	1	0.929*	0.936*	0.832*	0.753*	0.758*	0.643
4	-0.15	-0.01	0.929*	1	0.922*	0.839*	0.716	0.881*	0.514
5	-0.32	0.012	0.938*	0.922*	1	0.892*	0.663	0.669	0.526
6	-0.38	-0.04	0832*	0839*	0.892*	1	0.486	0.637	0.681
7	0.095	-0.10	0.753*	0.716	0.663	0.486	1	083*	0.331
8	0.253	-0.03	0759*	0.831*	0.669	0.637	0.83*	1	0.378
9	-0.53	-0.65	0643	0.514	0.526	0.681	0.381	0.379	1

Table-6. Correlation Coefficients Of Dizygotic Variables

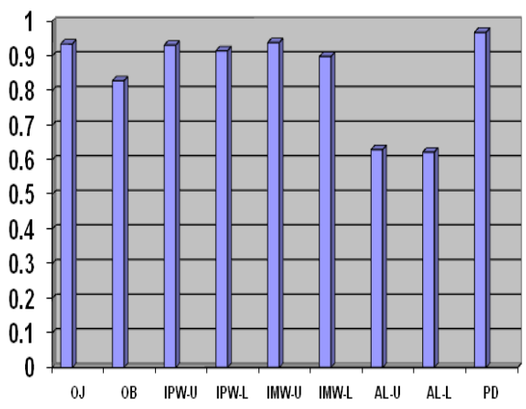
	1	2	3	4	5	6	7	8	9
1	1	0.491	-0.66	-0.09	-0.04	0.155	0.346	0.005	0.089
2	0.491	1	-0.58	-0.10	-0.41	0.406	-0.24	-0.43	0.063
3	-0.66	-0.58	1	0.189	0.775*	0.154	0.293	0.19	-0.21
4	-0.09	-0.10	0.189	1	0.457	0.05	0.199	0.23	0.279
5	-0.04	-0.41	0.775*	0.457	1	0.325	0.389	0.173	-0.12
6	0.155	0.406	0.154	0.05	0.325	1	0.431	0.291	-0.32
7	0.346	-0.24	0.293	0.199	0.389	0.431	1	0.633	0.015
8	0.005	-0.43	0.19	0.23	0.173	0.291	0.633	1	0.022
9	0.089	0.063	-0.21	0.279	-0.12	-0.32	0.015	0.022	1

1. **Over Jet**
2. **Over Bite**
3. **Inter premolar width(upper)**
4. **Inter premolar width (lower)**
5. **Inter molar width(upper)**
6. **-Inter molar width (lower)**
7. **Arch length (upper)**
8. **Arch length (lower)**
9. **Palate depth**



Graph-1. Mono and Dizygotic mean variance of twins

X- PARAMETERS Y- MEAN VARIANCE (mm)



Graph-2- Heritablility of the traits

X- PARAMETERS Y- HERITABILITY RATIO

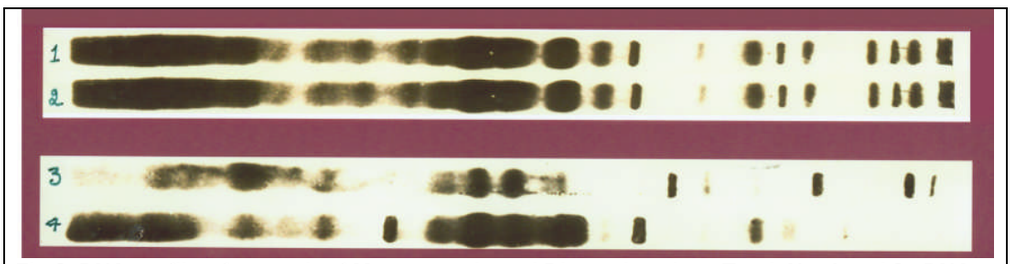


Fig.2. DNA profile of one pair of Mono zygotic and Dizygotic twins . Track No. 1 and 2: DNA profile of the identical twins. Track No. 3 and 4: DNA profile of Dizygotic twins .

and arch length of the both the arches. Similarly maxillary arch length is positively correlated with mandibular arch length. The correlation co-efficient of dizygotic twins shows significant positive correlations between Maxillary inter premolar width and maxillary inter molar width.

CONCLUSION

It has been decided to investigate the genetic and environmental influences on malocclusion commonly seen in our populations. 15 pairs of twins were selected in the study. zygocity was determined using DNA finger printing procedure. After the investigation the subjects were divided in to 8 monozygotic and 7 dizygotic twin pairs. Good U/L plaster models were made after taking alginate impressions. Nine parameters- Overjet, Overbite, Inter premolar width (Upper and Lower), Inter molar width (Upper and Lower), Arch length (Upper and Lower), Plate depth were made and recorded. Intra pair differences were calculated for each parameters in mono and dizygotic twin pairs and mean intra pair variance of each parameter was calculated. Using this data F ratio, Heritability Value (H), variance of Heritability V(H), standard Error SE (H) also calculated. A significant heritability values were obtained for seven out of nine parameters studied, overjet, overbite, both upper and lower inter premolar width, both upper and lower inter molar width, palate depth were showing a significant genetic variability where as upper and lower arch length showed insignificant genetic influence, indicating environmental influences substantially.

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