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"Comparative Evaluation of Craniofacial Morphology between Monozygotic and Dizygotic Individuals and Zygosity determination by Microsatellites -A Dermatoglyphic Study"

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ABSTRACT

Introduction: The interplay between genetic and environmental factors in craniofacial growth and development has long been a subject of research. This study aims to evaluate craniofacial morphology differences between monozygotic and dizygotic twins and determine zygosity using microsatellites and dermatoglyphics.

Materials and Methodology: A total of 33 pairs of twins (aged 16–26 years) from Chennai were analyzed. Zygosity determination was performed using microsatellites as DNA markers and fingerprint dermatoglyphic patterns. Craniofacial morphology was assessed through cephalometric analysis. Statistical evaluation was conducted using Pearson's correlation and independent t-tests.

Results: Monozygotic twins exhibited a significantly higher genetic influence on vertical craniofacial parameters compared to dizygotic twins. Dermatoglyphic analysis showed that total numerical fingerprint values were more reliable in determining zygosity than ridge counts. Microsatellite markers provided a 98% accuracy rate in zygosity determination.

Conclusion: This study confirms that genetic factors strongly influence craniofacial morphology, particularly vertical dimensions. Microsatellite DNA markers are a highly reliable method for zygosity determination, while dermatoglyphic patterns offer supplementary insights. The findings have important implications for Orthodontic treatment planning by identifying heritable skeletal characteristics.

Keywords: *Twins, Monozygosity, Dizygosity, Cephalometrics, DNA markers.*

1. INTRODUCTION

The influence of genetic and environmental factors on growth and development of the dentofacial complex has been the topic of debate and controversy from ancient times till date[1]. Numerous genetic epidemiology studies have attempted to explain how and why disease has a familial distribution[2]. Knowing more about the relative contributions of environment and genes to dentofacial and occlusal factors can further increase our understanding of the etiology of orthodontic diseases and consequently, the potential benefits and drawbacks of orthodontic treatment options[3]. Numerous investigations have demonstrated elevated heritabilities for the majority of facial and dental parameters, with the vertical parameters exhibiting greater genetic control in comparison to the horizontal ones. [4] Occasionally, studies involving families and twins have demonstrated the important influence of environmental factors. [5] Given that genetic and environmental factors can affect

variations in the size and shape of the skull and teeth, the twin method is among the most efficient ways to study genetically determined variables in orthodontics and other medical specialties. [6] The premise of twin studies is that any differences in

phenotype between the twins should be the result of environmental influences, or of identical genes interacting with different environmental factors[7]. Twins are unique individuals who offer a multitude of information, including and illuminating

insights into the mechanism of human craniofacial growth and development.There are 2 types of twins, Monozygotic or identical twins who originate from one fertilized egg and are identical in genetic makeup and sex and Dizygotic or fraternal twins who originate from 2 separate fertilized eggs and have a different composition[8] .Finding out for sure if a twin is identical or fraternal is called Zygosity determination.This

study attempts to determine whether the fingerprints could play a role with certainity in determining a diagnosis of monozygosity or dizygosity[9]. There are several methods that determine twin type or Zygosity, such as Anthropologic, Serologic and Genetic markers[10]. However, these methods are more than occasionally inefficient and sometimes inaccurate, so there is still a need for a more practical and informative method in Zygosity determination. [11] Recently Dinucleotide repeats or short repeats (Microsatellites) as DNA markers are used, they are highly variable between individuals and offer a simple, fast and exact approach for Zygosity determination. India as a country is well documented to be multiethnic[12]. There have been very few Twin studies carried out in India till date, so this study is undertaken in Chennai population of Tamilnadu State ethnic groups to provide a valuable insight in determining Twin Zygosity and cephalometric parameters of the craniofacial region in twins.

The objectives of the study include,

1. To determine Zygosity of twins using Microsatellite analysis and dermatoglyphics.

2. To study the cephalometric parameters of the craniofacial region in monozygous and dizygous twins.

3. To compare the level of significance among the monozygous and dizygous twins based on genetic and environmental influences of the craniofacial parameters using cephalometrics.

2. MATERIALS AND METHODOLOGY

The sample for this study consisted of 33 pairs of twins both males and females with age groups ranging from 16-26 years. These samples were collected in and around Chennai city. Zygosity for these samples was determined by utilizing microsatellites as DNA marker and fingerprints (Dermatoglyphics). Before collecting the blood samples and fingerprints from the twin samples, informed consent was taken.

Selection criteria

Subjects for the study were collected from Chennai city

Twins were all above the age of 16 years

No history of previous orthodontic therapy

No systematic complications

No gross facial asymmetry

Materials used for finger prints (Dermatoglyphics):

Magnification lens -10 X magnification.

Finger print roller (Rubber) - 4 inch breadth with metallic handle

Finger print plate - 7 inches X 12 inches (Thermoplastic Sheet)

Finger print ink: -Black printer’s ink.

Procedure to record fingerprints (Fig.1)

Clean the roller and the slab

Place a small quantity of ink on the slab, then using the roller, spread out evenly into a thin film.

The fingers of the subject should be cleaned with a cloth moistened with methylated or petroleum spirit.

Place the finger on the inked slab. Roll the finger from one side of the finger nail towards the other end of the finger-nail. The inked finger is then similarly fully rolled in contact with the paper, so as to record the complete pattern.

Both the operator and the subject must stand in a natural and unstrained position. The subject should be at the side of the bench and the operator should be at the end so that they are at right angles to each other.

Plain and rolled impressions

Fingerprints are impressions of the inner surfaces of the fingers from the first joint to the tips. When a print is made by simple contact between the finger and a receptive surface it is called a PLAIN impression to record the finger impression in full from the ridged surface, it is necessary to roll the finger from one nail end to the other end. Because of the method adopted, the resultant impression is called a ROLLED impression. A record plain impression and rolled impression are shown in

Fingerprints patterns

On an examination of fingerprints, it shows well-defined groups by means of their geometrical formations(Table 1&2). There are four main fingerprint groups as follows.(Fig. III & IV)

Arches

Loops

Whorls

Composite patterns

Cardinal points

With the exception of the arch patterns and accidental patterns, other patterns viz loops, whorls, twinned loops and lateral pocket loops are either sub-divided by "Counting" or "tracing" the ridges between the cardinal points, or fixed points namely, the CORES and the DELTA's (Fig.IV)

Core: The core means the central point of the pattern. It is near the point where the innermost ridge re-curves. In loop and whorl patterns there is only one core. In twinned loops and lateral pocket loops there are two cores.

Delta: The delta, as its triangular structure occurs at the point where the ridges, flowing across the finger, separate to enclose the basic pattern. One delta is found in loop patterns and two deltas are found in whorls, twinned loops and lateral pocket loops. Accidental pattern may have more than two deltas.

Ridge Characteristics:

Tracing the courses of an individual ridge, some ridges end abruptly, while others fork and become two. Sometimes, limbs of such a fork join together again almost at once and form an "enclosure". Occasionally short independent ridges and "Spur" formations occur. These peculiarities are called RIDGE CHARACTERESTICS. The different types of ridge characteristics are:

Termination, or ending

Enclosure, or lake

Independent ridge or island

Spur

For comparing fingerprints, two vital parameters viz pattern type and ridge characteristics are to be taken for comparison. Examination of pattern type will speed up the comparison. If two prints belong to different patterns, it means that they are not identical with each other. Since the prints are basically different in patterns, further comparison based on ridge characteristics is not required. If a set of prints belong to the same type of pattern, it cannot be concluded whether they are identical or not identical, unless the comparison of ridge characteristics is made. Identification is based only upon the ridge characteristics are found in identical sequences (viz position and direction) in both the given impressions, it can also be assumed, without doubt, that the impressions are identical with each other and are made by the same finger of the same individual.

Ridge counts

Since the arch and Tented arch patterns do not have the cardinal points (i.e. core and delta), no ridge counts are possible for these two patterns.Ridge counts are given for the loop, whorl and composite patterns. In loops, the number of ridges intervening between the core and delta are to be counted. In whorls, which occur in right hand fingers, the ridges intervening between the core and left side delta are counted and whereas the whorls, which occur in left hand fingers the ridges intervening between the core and right side delta are counted. In twinned loops and lateral pocket loops there are two well defined loops called ascending loop and descending loop. In these two patterns, the number of ridges intervening between the core and the delta of the ascending loop are to be counted. Since the accidental patterns have more than two deltas, it is not considered for ridge counts.To study fingerprints in twins of monozygotic and dizygotic in this study, the services of an Addl. Supdt. Of police (fingerprints) Retd., who is an expert and proficient in the field of Dactyloscopy is utilized in recording the fingerprints of 33 pairs of twins and classifying purpose "Henry System of Classification" was adopted. For the purpose of study the pattern type, numerical value and ridge counts were taken.

Pattern type

Table 1:The following different type of fingerprint patterns are denoted by the symbol noted against each. (Table. Below)

S.No	Pattern type	Subdivision	Symbol
1.	Arches	Plain Arches Tented Arches	A T
2.	Loops	Radical Loops Ulnar loops	R U
3.	Whorls	-	W
4.	Composite	Twinned Loops Lateral pocket loops Accidentals	TL LP AC

Numerical value

Table 2: The various fingerprint patterns are given numerical value .

Pattern type	Numerical value
Arch	1
Tented Arch	2
Radial Loop	3
Ulnar Loop	4
Whorl	5
Twinned Loop	6
Lateral Pocket loop	7
Accidental	8

Microsatellite analysis;

Microsatellites: These are short sequence of nucleotide (such as GATA) which are repeated over and over again a number of times in tandem. Microsatellites are genetic markers, these are highly polymorphic, and shows high levels of accuracy, and are used to identify unique individual. So these microsatellites are used are genetic markers in establishing zygosity of twins.

Micro satellite analysis include the following steps,
DNA isolation from twin samples
Quantification of DNA. (Spectrophotometer)
Polymerized chain reaction(PCR) using 9STR loci
PCR machine-Gene Amp 9700
PCR kit-AmpF/STR Profilier Plus kit
Gene scan analysis of PCR amplicons using automated DNA sequencer (ABI Prism 3700)
Genotyping
Materials used for DNA isolation:
Falcon tubes-Tarson-50ml and 15ml
Centrifuge-Remi R8C
Eppendroff tubes 1.5ml
Micro Pipettes-P 1000,200,100.
Reagents used for DNA Isolation
Reagent-A
Sucrose 109.54 gms
1 M Mgcl2 5.0 ml
Triton X 10.0 ml
1M Tris-HCL(PH-8) 10.0 ml
Make up to 1000ml with double distilled water.(DDW)
Reagent:-B
1M Tris-Hcl (pH-8) 40 ml
0.5 M Na-EDTA 12 ml

1 M Nacl 15 ml
Make up to 95 ml with DDW.
Autoclave.
Then add 5 ml 20% SDS (1%)
Reagent C:
5 M Na-per chlorate (MERCK) 100gms
Make up to 142 ml with DDW
Procedure for DNA isolation:
To the blood sample, add 4 volumes of reagent A in a Polypropylene tube. Mix gently till the solution becomes clear.
Centrifuge at 2500 rpm for 5 minutes to obtain a pellet, free from RBC. The supernatant containing lysed RBC is discarded carefully
Disturb the pellet thoroughly and add half the volume (as that of blood sample) of Reagent B. Mix thoroughly and gently by inverting for 3-4 minutes till the solution becomes viscous.
Add reagent C (1/4th volume of reagent B) and mix gently for 3-4 minutes
Add equal volumes (as that of reagent B + C) of phenol and chloroform. Mix well and centrifuge at 2500-3000 rpm for 7-8 minutes to separate into 3 layers viz, aqueous layer, protein layer and solvent layer.
Transfer the aqueous layer carefully into another centrifuge tube using a broad mouth tip (Care should be taken that the protein layer is not distributed)
Add equal volumes of chloroform to the supernatant and mix gently for a minute and centrifuge at 2500 rpm for 5 minutes
Transfer the aqueous phase to a fresh tube.
Add equal volumes of chilled isopropyl alcohol and mix gently to precipitate the DNA
Spool out the DNA lump in a fresh Eppendorf tube and decant alcohol.
Wash the DNA twice with 70% alcohol and give a short spin to remove alcohol
Dry the pellet properly and ensure the whole alcohol is dried.
Dissolve the pellet in 50-100 micro litre of TE
Incubate at 55 degree for 45 min to enhance the dissolution
Store the DNA samples at 4 degrees.

Quantification of DNA:

Optical density (using Spectrophotometer) Absorption spectrum of DNA between 260-280nm. At 260nm and absorption of 1.00 O.D measured in a cuvette with 1 cm path length is indicative that concentration of DNA is approximately 50 micrograms/ml.The ratio of absorption at 260nm indicates the purity of the sample. O.D of DNA solution should range from 1.7-1.8.

Gel Electrophoresis:

Agarose gel electrophoresis is an efficient technique to separate DNA molecule according to their molecular weight in the same manner as a sieve. The gel tray and combs were cleaned with methanol.The open ends of the gel tray ware sealed with tape, comb was placed properly 0.8 gm of agarose was dissolved, in 100ml 0.5 X TAE buffer in a 250ml. Conical flask and was boiled in microwave oven to dissolve agarose. Completely.0.7 microliters ethidine bromide was added from the stock to make a final conc. Of 0.5micrograms/ml. Gel was cooled at 60 degrees and poured onto a gel tray and was allowed to cool and set.After the gel was completely set, tapes were removed from the sealed ends and comb was removed carefully. 0.5 X TAE buffer was added to electrophoretic tank to cover the gel to the depth of 1 cm. DNA diluents was taken and mixed with 1 microlitre of 6X loading dye. The mixture was then loaded carefully into the casted gel.The stranded DNA was also loaded along with the sample to quantify DNA and electrophoresis was carried out at a constant voltage of 80V. After the dye had run halfway the gel was run under UV light of Trans illuminator and photographed.

Polymerized chain reaction (PCR) : PCR is carried out using 9STR loci-(PCR machine used -Gene Amp-9700). The kit used for PCR was AmpFISTR profilier Plus kit.

Steps	Conditions
Initial Denaturation	950C 11 min
28 cycles at	940C 1 min
590C 1 min	
720C 1 min	
Final Extension	600C 45 min
Hold at	250C Forever

As the final step genotyping was done to distinguish between monozygotic and dizygotic twins.

In the genotyping all the 9 loci are dye labelled according to their size range (base pairs). In establishing the zygosity if the given pair is monozygotic all the loci should match which is represented in the form of waves (base pair lengths) in case of dizygotic twins all the loci do not match.

In our study all the lateral cephalograms were traced manually. The study was undertaken to assess the heritable parameters in monozygous and dizygous twins based on vertical and horizontal skeletal characteristics.

Data files of the landmarks were used to compute the following cephalometric variables.

Total anterior facial height measured from Nasion to Menton (N-Me)

Upper anterior facial height measured from Nasion to anterior nasal spine (N-ANS)

Lower anterior facial height measured from anterior nasal spine to menton (ANS-Me).

Total posterior facial height measured from sella to gonion (S-G0).

Saddle angle –angle Nasion, sella, and articulare (N-S-Ar).

Articular angle –angle between sella, articulare and gonion(S-Ar-Go).

Gonial angle –angle between articulare and tangent to the mandibular base.

Sum of the posterior angles

Maxillary length measured from ANS-PNS

Mandibular length Measured from gonion to Gnathion.

Jarabak Ratio: Posterior facial height to anterior facial height.

At the time of study twins were given identification number from 1-66. The monozygotic twins (according to the patient number) 1-4,9-12,17-18,25-26,29-36,39-40,43-44,47-48,57-58,59-60,63-64,65-66.The dizygotic twins (according to the patient number) 5-8,13-16,19-24,27-28,37-38,41-42,45-46,49-56,61-62.

Statistical analysis:

For statistical analysis twins were divided into 2 groups, group 1 –monozygotic twins, and group 2-dizygotic twins within each group first individual is grouped under subgroup 1 and the second individual is grouped under subgroup 2.

Mean and standard deviation for each of the above mentioned parameters were calculated for both mono and dizygotic twins by SPSS version 4.0. Pearson product moment correlation coefficients were then calculated. To find out the correlation between the subgroups, and students independent‘t’ test was done to compare between the 2groups monozygotic and dizygotic.

3. RESULTS

This study was done to compare the level of significance among the monozygous and dizygous twins based on genetic and environmental influences of the craniofacial parameters using cephalometrics.

Dermatogyphic analysis:

The analysis of the fingerprints of the 33 pairs of twins was done taking into account the pattern type, Digital numerical value and total numerical value and Digital ridge counts and total ridge counts which are summarized under the table 3 and 4 for monozygotic and dizygotic twins respectively. The total numerical value and ridge count for T1 was 40 and 100 and T2 was 38 and 76 of monozygotic pair whereas for dizygotic twins the total numerical value and total ridge count was 46 and 166 for T1 and 37 and 62 for T2 respectively.

Table 3: Monozygotic twin pair

	Pattern type					Digital numerical value					Digital ridge count					Total Numerical value	Total Ridge counts
T1	U	U	U	U	U	4	4	4	4	4	14	6	9	8	15	40	100
	U	U	U	U	U	4	4	4	4	4	9	10	6	9	14		
T2	U	U	U	U	U	4	4	4	4	4	10	2	5	9	14	38	76
	U	T	U	U	U	4	2	4	4	4	9	0	2	9	16		

Table 4: Dizygotic twin pair

	Pattern type				Digital numerical value					Digital ridge count						Total Numerical value	Total Ridge counts
T1	W	W	U	W	U	5	5	4	5	4	20	12	16	19	16	46	166
	U	W	W	W	U	4	5	5	5	4	16	15	18	18	16		
T2	U	U	U	U	U	4	4	4	4	4	6	3	2	14	9	37	62
	U	U	A	U	U	4	4	1	4	4	8	4	0	5	11		

Table 5: Monozygotic Twins:

Variables	SG1	SG2	P value
N-Me	120.9±9.5	120.9±8.6	1.0
N-ANS	52.5±4.2	53.3±4.0	0.12
ANS-Me	68.5±7.6	67.6±6.9	0.24
S-GO	85.2±8.9	84.6±9.6	0.52
NSAr	124±3.8	125.8±4.3	0.20
SAr-GO	145.1±5.8	146.4±6.3	0.38
Ar-GO-Gn	121.3±6.3	121.9±6.9	0.49
Sum	393.8±5.3	394.1±5.1	0.49
ANS-PNS	55.5±4.0	56.3±4.2	0.49
GO-Gn	80.2±5.5	79.0±5.8	0.03
PFH/AFH	70.4±2.7	70.2±3.9	0.74

Table 6 : Pearson's product moment correlation coefficients for monozygotic twins

VARIABLES	CORELLATION COEFFICIENT	
	r value	p value
N-Me	0.92(+++)	<0.0001
N-ANS	0.88(++)	<0.0001
ANS-Me	0.93(+++)	<0.0001
S-GO	0.94(+++)	<0.0001
NSAr	0.72(+)	0.06
SAr-GO	0.78(++)	0.02
Ar-GO-Gn	0.86(++)	0.001
Sum	0.94(+++)	0.0001
ANS-PNS	0.7(+)	0.001
GO-Gn	0.80(++)	0.0001
PFH/AFH	0.76(++)	<0.0001

Karl Pearson's Classification

Perfect positive correlation(++++)

0.9-1 Very high degree correlation (+++)

0.75-0.9 Significant high degree correlation (++)

0.6-0.75 Moderate degree correlation (+)

The overall facial height for SG1 and SG2 among monozygotic twins was found to be 120.9 ±9.5 & 120.9 ±8.6 as mean values. Comparison between the 2 subgroups Pearson's correlation coefficient for TAFH was found to be 0.92 which shows a very high degree of correlation. (table 5,6)

2. The upper anterior facial height for SG1 and SG2 among monozygotic twins was found to be 52.5±4.2 and 53.3±4.0 as mean values. Comparison between the two subgroups Pearson's correlation coefficient for UAFH was found to be 0.88 indicating significantly high degree of correlation

3. The lower facial height for SG1 and SG2 among monozygotic twins was found to be 68.50±7.60 and 67.6±6.9 as mean values. Comparison between the 2 subgroups, Pearson's correlation coefficient for PFH was found to be 0.93 referring to a very high degree of correlation.

4. Posterior facial height for SG1 and SG2 among monozygotic twins was found to be 85.2±8.9 and 84.6±9.6 as mean values. Comparison between the 2 sub groups, Pearson's correlation coefficient for PFH was found to be 0.94 suggesting a very high degree of correlation.

5. Saddle Angle-for SG1 and SG2 among monozygotic twins was found to be 124.3±3.8 & 125.8±4.3 as mean values. Comparison between the two sub groups, Pearson's correlation coefficient for Saddle Angle was found to be 0.72(r value) indicating moderate degree correlation.

6. Articular Angle - for SG1 and SG2 among monozygotic twins was found to be 145.1±5.8 and 146.4±6.3 as mean values .Comparison between the two sub groups, Pearson's correlation coefficient for Articular angle was found to be 0.78 suggesting significantly high degree correlation showing significant high degree correlation.

7. Gonial Angle-for SG1 and SG2 among monozygotic twins was found to 121.3±6.3 and 121.9±6.9 as mean values. Comparison between the two sub groups, Pearson's correlation coefficient for Gonial angle was found to be 0.86 showing significant high degree correlation.

8. Sum Angles-for SG1 and SG2 among monozygotic twins was found to be 393.8±5.3 & 394.1±5.1 as mean values. Comparison between the two sub groups, Pearson's correlation coefficient for sum angle was found to be 0.94(r value) suggesting a very high positive correlation.

9. Maxillary Length-for SG1 and SG2 among monozygotic twins was found to be 55.5±4.0 and 56.2±4.2 as mean values. Comparison between the two sub groups, Pearson's correlation coefficient for Maxillary Length was found to be 0.7 (r value) showing moderate degree correlation.

10. Mandibular Length-for SG1 and SG2 among monozygotic twins was found to be 80.2±5.5 and 79.0±5.8 as mean values. Comparison between the two sub groups, Pearson's correlation coefficient for Mandibular Length was found to be 0.80 (r value) indicating significant high degree correlation.

11. Jarabak Ratio-for SG1 and SG2 among monozygotic twins was found to be 70.4±2.7 and 70.2±3.9 as mean values. Comparison between the two sub groups, Pearson's correlation coefficient fot Jarabak Ratio was found to be 0.76 suggesting moderate degree correlation.

Dizygotic twins

Table 7: Mean and Standard deviation forSG1 and SG2 in Group II

Variables	SG1	SG2	P value
N-Me	114.4±8.1	111.6±8.5	0.07
N-ANS	50.1±3.1	49.4±3.6	0.31
ANS-Me	64.3±6.1	62.2±7.0	0.16
S-GO	76.4±5.9	76.6±5.7	0.89
NSAr	123.1±6.8	125.6±6.1	0.82
SAr-GO	145.1±6.8	144.4±7.0	0.70
Ar-GO-Gn	124.4±5.2	123.7±4.5	0.86
Sum	394.5±6.1	394.7±6.2	0.86
ANS-PNS	54.7±3.6	53.9±3.6	0.60
GO-Gn	76.6±5.9	75.6±5.8	0.37
PFH/AFH	66.2±3.7	68.0±4.8	0.18

Table 8: Pearson's product moment correlation coefficient for dizygotic twins

VARIABLES	CORELLATION COEFFICIENT

	r value	p value
N-Me	0.76(++)	<0.0001
N-ANS	0.65(+)	0.003
ANS-Me	0.64(+)	0.004
S-GO	0.32	0.12
NSAr	0.70	0.001
SAr-GO	0.40	0.06
Ar-GO-Gn	0.20	0.15
Sum	0.83(++)	0.0001
ANS-PNS	0.60	0.003
GO-Gn	0.73(+)	0.001
PFH/AFH	0.27(+)	0.16

The overall facial height-for SG1 and SG2 among dizygotic twins was found to be 114.4±8.1 and 111.6±8.5 as mean values. Comparison between the two sub groups, Pearson's correlation coefficient for TAFH was found to be 0.76 suggesting significant high degree correlation.(table 7,8)

The upper anterior facial height-for SG1 and SG2 among dizygotic twins was found to be 50.1 ±3.1 and 49.4±3.6 as mean values. Comparison between the two sub groups, Pearson's correlation coefficient for UAFH was found to be 0.65 indicating moderate degree correlation.

The anterior lower facial height-for SG1 and SG2 among dizygotic twins was found to be 64.3±6.1 and 62.2±7.0 as mean values. Comparison between the two sub groups, Pearson's correlation coefficient for LAFH was found to be 0.64 showing moderate degree correlation.

4. The total posterior facial height-for SG1 and SG2 among dizygotic twins was found to be 76.4±5.9 and 76.6±5.7 as mean values. The Saddle Angle-for SG1 and SG2 among dizygotic twins was found to be 123.6±6.8 and 125.6±6.1 as mean values.The Articular Angle-for SG1 and SG2 among dizygotic twins was found to be 145.1±6.8 and 144.4±7.0 as mean values. 7. The Gonial Angle-for SG1 and SG2 among dizygotic twins was found to be 124.4±5.2 and 123.1±4.5 as mean values. The Maxillary length for SG1 and SG2 among dizygotic twins was found to be 54.7±3.6 and 53.9±3.6 as mean values. Pearson's correlation coefficient for Maxillary Length was found to be 0.60. The comparison between these parameters between subgroups did not show any correlation.

The Sum Angle-for SG1 and SG2 among dizygotic twins was found to be 394.5±6.1 and 394.7±6.2 as mean values. Comparison between the two sub groups, Pearson's correlation coefficient for Sum Angle was found to be 0.83 indicating significant high degree correlation.

The Mandibular Length-for SG1 and SG2 among dizygotic twins was found to be 76.6±5.9 and 75.6±5.8 as mean values. Comparison between the two sub groups, Pearson's correlation coefficient for Mandibular Length was found to be 0.73 suggesting moderate degree correlation.

Jarabak Ratio-for SG1 and SG2 among dizygotic twins was found to be 66.2±3.7 and 68.0±4.8 as mean values. Comparison between the two sub groups, Pearson's correlation coefficient for Jarabak Ratio was found to be 0.27 showing moderate degree correlation.

4. DISCUSSION

In this study 11 variables of the dentofacial complex were analyzed statistically to assess heritability of dentofacial complex. It was observed that all the parameters analyzed were having correlation co-efficiency between 0.27 and +1 which is the maximum comparing monozygotic and dizygotic twins. This shows that the variables were under strong influence of heredity.

In case of monozygotic twins,

Total Anterior Facial Height (N-Me) was found to be under strong hereditary influence with significant high degree of correlation (0.92 - r value) with very weak component of environmental influence. This is in accordance with the findings of Dudas and Sasssouni from their longitudinal study of mandibular growth. This is also in accordance with Arya et al [5]who reported a genetic variability of 62.5% for anterior facial height.

Upper Anterior Facial Height (N-ANS) was found to have strong hereditary influence (0.88-r value). This shows that there is minor component of environmental influence. This variable was investigated by Claudio Manfredi and Roberto Martina[13]. This is also is accordance with Hunter and Lundstrom[14] who found higher heritable values among vertical

compared with horizontal variables. They found high coefficient values for TAFH and LAFH and low correlation for upper facial length. From the therapeutic point of view these results indicate that there are more chances of success in orthopaedic treatment of the posterior facial complex and weak chances to influence the upper anterior portion of face.

Lower Anterior Facial Height (ANS-Me) was found to be under strong hereditary influence (0.93-r value) with a very weak component of environmental influence. This is in accordance with Hunter and Lundstorm [14] who found higher correlation values for TAFH and LAFH. This is also in agreement with Lundstrom and Mc William[15].This suggests little chance to modify skeletal vertical dimensions of lower third of face (Strong Genetic Control).

PFH-was found to have high degree of hereditary influence with correlation coefficient of 0.94 – r value with a significant component of genetic influence. This variable was investigated by Claudio M. and Martina [13] they found a correlation coefficient of 0.94, suggested a very high degrees of correlation coefficient and strong genetic influence.

Saddle Angle- correlation coefficient for Saddle Angle was found to be 0.72 showing moderate degrees of correlation. Indicating that shape of Cranial base was under strong influence of hereditary. This also indicates a moderate component of environmental influence. This variable was investigated by Manfredi and Martina [13] who found correlation coefficient of 0.93 suggesting a very high degree of correlation coefficient and Strong genetic influence.

Articular angle – Correlation coefficient for Articular Angle was found to be 0.78 indicating a significant high correlation-suggesting a high degree of strong genetic influence, indicating that the forward and backward diversion of mandible during growth is under strong influence of hereditary. This also shows that there is an environmental influence on these parameters. Correlation coefficient for Articular Angle according to study done by Manfred and Martina[13]was 0.65 shows only moderate degree of correlation.

Gonial Angle – was found to have highest correlation of 0.86 indicating that this parameter is under very strong genetic influence with very weak environmental influence. This reveals that the shape of mandible is under strong influence of hereditary. This is in agreement with the study done by Manfred and Martina[13]whose correlation was 0.87.

Sum Angles – Correlation coefficient for sum angles was 0.94 indicating that it is under very strong genetic influence with very weak environmental influence.

Maxillary Length – Correlation coefficient for maxillary length was 0.7 indicating that it is under (moderate degree correlation) moderate zygotic influence with a component of environmental influence. This parameters studied by Lobb[16].

Mandibular Length – was found to be under strong hereditary influence compared to maxillary length with lesser component of environmental influence. This is in agreement with result of Lobb[16] who found mandible is more variable than maxilla and cranial base.

Jarabak Ratio – Correlation coefficient was found to be 0.79 showing significant high degrees of correlation. The mean and S.D are in accordance with horizontal growth pattern among twins. The rotation of the mandible is i.e. anticlockwise direction.

Dizygotic

Total anterior Facial Height (N-Me) correlation coefficient for TAFH was 0.76 – r value. We have found that TAFH was under significant genetic influence. This shows that there is a minor environmental influence on TAFH. This variable was investigated by Manfredi and Martina[13] – they found a correlation of 0.19 (s). This indicates the greater component of environmental influence than genetical influence. This parameter was also studied by Dudas and Sassouni [17] who showed no difference exists between growth of monozygotic and dizygotic for anterior facial height. In our study we found correlation coefficient for anterior facial height in dizygotic twins was less when compared to monozygotic twins indicating that environmental influence was more in dizygotic twins than monozygotic twins.

Upper anterior facial height – correlation coefficient for UAFH was found to be 0.65. This shows that genetic influence has a weak role to play. This also shows that there is major component of environmental influence on UAFH. This is in accordance with Manfredi and Martina who found a correlation of 0.69 which is significant.

Lower anterior facial height : Correlation coefficient for LAFH was found to be 0.64 having moderate hereditary influence, with a significant component of environment influence. This parameter was studied by Manfredi and Martina[13] and found that there is a very significant environment influence on the ALFH, they got the correlation coefficient of 0.16.

Total posterior facial height: Correlation coefficient 0.32 we found that there is a very significant environmental influence on the TPFH. On contrary to this Manfredi and Martina[13] had got the correlation coefficient of 0.77 indicating a significant genetic influence.

Saddle angle: Correlation coefficient0.70. We found that there is a weak genetic and moderate environment influence on the saddle angle according to the study of Manfredi and Martina[13], the saddle angle had a moderate genetic and weak

environmental influence with correlation coefficient of 0.78.

Articular angle : (correlation coefficient 0.40) We found that there is a weak genetic and strong environmental influence on the articular angle. This parameter is in accordance with Manfredi and Martina[13] who got a correlation coefficient of 0.44.

Gonial angle: (correlation coefficient 0.20) we found that there is a very significant environmental and very weak genetic influence on the gonial angle. The same parameter was studied by Manfredi and Martina[13]both these correlation coefficients were non significant.

Sum angle (correlation coefficient 0.83) we found that there is a very significant genetic , and weak environmental influence on the sum of the posterior angles.

Maxillary length: (correlation coefficient 0.600) we found that there is a moderate genetic influence, and significant environmental influence on the maxillary length.

Mandibular length: (correlation coefficient 0.73) we found that there is a significant genetic and weak environmental influence on the mandibular length. This parameter was studied by Lobb [17]and is in accordance with their study. He found that mandible was more variable than maxilla and cranial base.

Jarabak ratio:(Correlation coefficient -0.27)we found that there is a mild genetic and environmental influence on the jarabak ratio. This is also in accordance with horizontal growth pattern as seen in MZ twins.

A basic problem in twin research is the reliability of twin diagnosis. The present study was focused on the zygosity determination by microsatellites dermatoglyphic procedures, as well as the assessment of genetic and craniofacial characters of monozygotic and dizygotic twins. Twins studies may be used to compare different variables with regard to their dependence on heredity and environment.

Using dermatoglyphics an orthodox method of determination of zygosity brought out absolute differences and variance among the twins. It is found that the total numerical values in monozygous pairs are either similar or in variation between 1-7.5%. Whereas in dizygous pairs the variation is between 10-22%. In the area of total ridge counts the variation in monozygous pairs is between 0.8-25%. Whereas in dizygous pairs the variation goes between 5-87%.

The comparative study over the total numerical value in both monozygous and dizygous pairs reveal that they are within the flexible parameter. In the area of total ridge counts both monozygotic and dizygotic pairs did not have flexible parameters. Hence, total ridge count may not be very useful in determining the pair whether they are monozygotic or dizygotic.

We have used microsatellites as DNA marker which is largely automated, rapid and efficient method for determining twin zygosity.

We typed 33 pairs of twins, and on zygosity determination revealed 17 pairs of monozygotic and 16 pairs of dizygotic twins. With 9 STR markers the probability that any twin pair was monozygous , if all the markers were concordant was 98%.

If zygosity determination with DNA markers, or blood group typing for all subjects is not feasible, rather than using classification indices based on other studies and optimal classification scheme can be achieved by using a zygosity questionnaire of which the reliability and validity of the questions can be established.

Variability observed in the craniofacial skeleton must undoubtedly have some effect on the facial skeleton that contains various skeletal elements making up the craniofacial complex.

As an orthodontist deals with both skeletal base and the alveolar bone during the course of treatment, he has a major role in designing the future shape of the bony components of craniofacial components as revealed in two dimensional cephalogram.

This study of twins identifying craniofacial patterns on cephalometrics revealed the evidence of heritability more stronger (0.93-r value) for monozygous twins and a weak correlation of environmental influence. Among dizygous twins the genetic influence was only moderate (0.76-r value) and a strong influence of environment was seen.

The results of our study evaluated that the vertical and horizontal parameters are under a strong genetic control, but when compared to the horizontal parameters, the vertical parameters showed a very significant genetic influence. Out of the vertical parameters evaluated in monozygous twins the lower anterior facial height showed a very strong correlation indicating that it is under strong genetic influence. Monozygous twins, vertical parameters seem to have similar genetic influence between anterior and posterior variables and in dizygotic twins the vertical parameters seen to be differing with more correlation anteriorly (0.76) than posteriorly (0.32).

The maxillary and mandibular lengths seems to be more genetically determined in monozygous and dizygous twins. But among the maxillary and mandibular lengths, mandibular length seemed to have strong genetic influence than environmental influence on the growth of lower face.The growth pattern, that is inclusive of 3 cranial angles has a very strong genetic influence (0.94 MZ, 0.83 DZ) revealing that the lower half of face has a dominant role during growth of orofacial complex hence causing a horizontal growth of the mandible.The Jarabak ratio which is indicative of growth rotation reveals that in monozygotic twins there is strong genetic influence bringing about counterclockwise roatation of the mandible, influencing

the overall facial height. In dizygous twins the growth rotation reveals a weakly correlated genetic influence and more of environmental influence bringing about counterclockwise rotation of the mandible.

5. SUMMARY & CONCLUSION

The twin method is one of the most effective methods available for investigating genetically determined variables in orthodontics as well as in other medical fields.

1.Microsatellites as DNA markers revealed 98% concordance with zygosity determination and found to be the most accurate, rapid, efficient and largely automated procedure for zygosity determination.

2. Dermatoglyphics: The total ridge count did not have a flexible parameter for both monozygotic and dizygotic twins. Considering above points of validation it is concluded that the pairs of monozygous and dizygous can be differentiated by studying the total numerical value of their fingerprints, rather than their total ridge counts. The dermatoglyphic study among 33 pairs studied revealed that 19 pairs were monozygotic and 14 pairs were dizygotic.

The heritability of craniofacial characteristics revealed that cranial base is also under strong genetic influence. There is a high genetic determination for the vertical and horizontal parameters, among these parameters vertical parameters showed a significantly high genetic influence especially for the lower anterior facial height among monozygous twins. In dizygous individuals the vertical parameters are comparatively less genetically influenced than monozygous twins. The lower third of face in monozygous individuals as said earlier has a very strong genetic control, which means from clinical stand point a minimal chance to achieve stable vertical modification.

Conflicts of interest: The authors donot have any conflict of interest.

REFERENCES

[1] Akane A, Matsubara K, Shiono H, Yamada M, Nakagome Y: Diagnosis of twin zygosity by hyper variable RFLP markers. Am.J.Med Genet 1991 ; Oct 1:41(1):96-8.

[2] Albert. Becker M.S Andreas Busjahn,Hans-Dieter F, Sylvia B, James Herbert S, Friedrich. Twin zygosity. Automated determination with microsatellites. J. Reprod Med 1997, May,42(5):260-6.\

[3] Allen G., Diagnostic efficiency of fingerprint and blood group differences in a series of twins. Acta Genet. 1968;17: 359-374.

[4] Arya B.S, Savara B.S, Clarkson Q.D, and Thomas: -Genetic variability of craniofacial dimentions Am.J.Orthod 1973;43;207-215

[5] Boklage C.E: Developmental differences between singletons and twins in distributions of dental diameter asymmetries. Am. J. Phys. Anthropol: 1987;74;318-31.

[6] Bonnelykke B, Haug M, and Kristofferesen K, Gurtler H:Evaluation of zygosity diagnosis in twin pairs below age 7 by means of a mailed questionnaire. Acta Genet Med Gamellol (Roma);1989;38;(3-4):305-13

[7] Boraas JC, Messer LB, Till MJ: A genetic contribution of dental caries, occlusion and morphology as demonstrated by twins reared apart. J. Dent Res 1988;Sep;67(9);1150-5

[8] Brismer "Dermantogyphics of twins: A study based on a new systematic". Acta Genetic Medicae et Gamellogiae, 1957; XVII.

[9] Brown T; Townsend.G.C; Richards L.C: A study of dentofacial morphology in South Australian twins. Aust. Dent. Journal. 1987;32;81-90

[10] Burke P.H., Intrapair facial differences in twins". Acta Genet 1989;38;37-47

[11] C Derom, E Bakker, R Vlietinck, R Derom, H Van den Berghe,M Thiery and P Pearson.Zygosity determination in new born Twins using DNA variants. Journal of Medical genetics.1985, vol 22, 279-82

[12] Chen WJ, Chang HW, Wu MZ, Lin CC, Chang C, Chiu YN, Soong WT: Diagnosis of zygosity by questionnaire and polymarker polymerase chain reaction in young twins". Behav Genet. 1999;-23Mar;29(2);115-23.

[13] Claudio.M, Martina.R, and G.B.Grossi:Heretability of 39 orthodontic cephalometric parameters on MZ,DZ and

MN paired singletons. Am.J.Orthod. 1997;1:44-51.

[14] Lundstorm. A and McWilliams J: Comparison of some cephalometric distances and corresponding facial proportions with regard to heritability. Eur. J or Orhod 1988: 10: 27-29.

[15] Lundstorm. A; McWilliam J.S : A comparison of vertical and horizontal cephalometric variables with regard to heritability: Eur. J. Orthod 1987:9:104-108.

[16] Lobb W.K. “Craniofacial morphology and occlusal variation in monozygous and dizygous twins” Angle Orthodont 1987;57;219-233

[17] Dudas M. And Sassouni V. “The heredity components of mandibular growth, a longitudinal study “Am.J.Orthod 1973;43;314-323