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CRANIOFACIAL SHAPE IN PATIENTS WITH BETA THALASSEMIA:

A MORPHOMETRIC ANALYSIS

PETROS ROUSSOS

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Supervisor of the Master's Thesis of Dr. Iosif Sifakakis

Three-Member Committee for examination of Master's Thesis:

1. Iosif Sifakakis

2. DemetriosHalazonetis

3. Mitsea Anastasia

ΕΘΝΙΚΟΝ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟΝ ΠΑΝΕΠΙΣΤΗΜΙΟΝ ΑΘΗΝΩΝ

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ΕΙΔΙΚΕΥΣΗ ΟΡΘΟΔΟΝΤΙΚΗ

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Τριμελής Επιτροπή για την Αξιολόγηση της Μεταπτυχιακής

Διπλωματικής Εργασίας:

1. Ιωσήφ Σηφακάκης

2. Δημήτριος Χαλαζωνίτης

3. Αναστασία Μητσέα

Το σχήμα του κρανιοπροσωπικού συμπλέγματος σε ασθενείς με β-θαλασσαιμία:

Μορφομετρική ανάλυση

Περίληψη

Σκοπός: Η αξιολόγηση με μορφομετρική προσέγγιση του σχήματος του κρανιοπροσωπικού συμπλέγματος σε ασθενείς με β-θαλασσαιμία και η σύγκριση τους με αντίστοιχους ασθενείς ελέγχου.

Υλικό και μεθοδολογία: Η ομάδα των ασθενών με β-θαλασσαιμία είχε 40 ασθενείς (24 άνδρες, 16 γυναίκες). Σε κάθε ασθενή με β-θαλασσαιμία έγινε αντιστοίχιση 2 ασθενών ελέγχου με κριτήριο το φύλο και την ηλικία (συνολικά 80 άτομα ελέγχου). Για κάθε ασθενή έγινε λήψη πλάγιας κεφαλομετρικής ακτινογραφίας (συνολικά 120 ακτινογραφίες). Σε κάθε ακτινογραφία πραγματοποιήθηκε ιχνογράφηση 15 καμπύλων και 127 ημισημείων. Στα παραπάνω έγινε αλληλεπίθεση Προκρούστη και Ανάλυση Κύριων Παραγόντων προκειμένου να περιγραφεί η ποικιλομορφία του σχήματος της βάσης του κρανίου, του μέσου τριτημορίου του προσώπου, της κάτω γνάθου και του κρανιοπροσωπικού συμπλέγματος για κάθε φύλο ξεχωριστά. Υπολογισμός σφάλματος έγινε με επανάληψη της ψηφιοποίησης και των μετρήσεων σε 20 ακτινογραφίες από δύο ερευνητές.

Αποτελέσματα: Η ομάδα της β-θαλασσαιμίας είχε διαφορά στο σχήμα του κρανιοπροσωπικού συμπλέγματος και για τα δύο φύλα ($P<0,001$). Οι υπό εξέταση ομάδες διαχωρίζονταν μεταξύ τους στο σχηματοχώρο. Οι κυριότερες διαφορές των ασθενών με β-θαλασσαιμία περιλάμβαναν μικρότερο σώμα της κάτω γνάθου, προβολή του μέσου τριτημορίου του προσώπου και μειωμένο οπίσθιο κάτω ύψος προσώπου. Τα παραπάνω ευρήματα συμφωνούν με τις ήδη υπάρχουσες παρατηρήσεις που βρίσκονται στη βιβλιογραφία

Συμπεράσματα: Η β-θαλασσαιμία προκαλεί σημαντικές μεταβολές στο κρανιοπροσωπικό σύμπλεγμα. Το σχήμα του προσώπου των ασθενών αυτών έχει την τάση να εμφανίζεται περισσότερο κυρτό και με πιο αποκλίνοντα σκελετικά επίπεδα σε σχέση με αυτό του φυσιολογικού πληθυσμού.

Craniofacial shape in patients with beta thalassemia:

A morphometric analysis

Summary

AIM: To morphometrically evaluate the shape of the craniofacial complex of patients with betathalassemia and compare it with matched controls.

MATERIALS AND METHOD: Beta thalassemia group consisted of 40 patients (16 females, 24 males). Each patient was matched on age and gender to two controls (n=80 controls). A total of 120 lateral cephalometric radiographs were collected, one from each patient, digitized and traced with 15 curves and 127 semi-landmarks. These landmarks were subjected to Procrustes superimposition and principal component analysis in order to describe shape variability of the cranial base, maxilla and mandible, as well as of the entire craniofacial complex for each sex. Error of the method was evaluated using both an intra- and interobserver approach.

RESULTS: The beta thalassemia group was significantly different in shape to the control group for both sexes ($P<0.001$). The groups were clearly separated in shape space. The main differences were related to smaller mandibular body for the thalassemia group, midface protrusion and decrease in posterior face height. These shape differences are in accordance with the well known clinical and radiological characteristics of this disease.

Introduction

Thalassemia syndromes are congenital haemolytic anaemias characterised by absent or reduced synthesis of one or more globin chains of haemoglobin. The decline in globin chain production leads to a decrease in production of functioning hemoglobin tetramers resulting in unpaired *α* and *β*chains that are incapable of properly releasing O_2[1]. The accumulation of unpaired chains disrupts the majority of the organ systems, leading to high mortality rate, if left untreated[2].

Thalassemias are divided into alpha and beta, depending on the affected gene. Beta thalassemias are classified as major, intermediate and minor, depending on the level of *β* chain production[1]. Clinical manifestations differ according to the beta thalassemia type, varying from minor morphologic abnormalities to life threatening conditions[3].

Previous studies on beta thalassemia patients used clinical examination or conventional cephalometric analysis. In addition to the inherent limitations of conventional cephalometrics[4], in beta thalassemia patients the evaluation of angles SNA and SNB may be misleading, since the bridge of the nose (point Nasion, which represents the anterior limit of the cranial base) is depressed[5].

Geometric morphometrics (GM) is the study of shape variation and its covariation with other variables[6]. It offers a quantitative way of comparing the anatomical features of species or individuals. Combined with modern statistical analysis methods it offers the possibility of

comparing the craniofacial pattern between two subjects, invariant to location, scale and orientation of the subjects[4].

Geometric morphometrics have been used to study shape differences between healthy controls and patients affected by major syndromes, such as Down's syndrome[7], fetal alcohol syndrome[8] , Glut-1Ds[9] , 22q11.2 deletion syndrome[10] , Apert's syndrome[11] , Pierre Robin sequence and Treacher Collins syndrome[12] as well as syndromic and nonsyndromic craniosynostoses[13] .

Beta thalassemia research, although advanced, is lacking a morphometric study of its effects on the craniofacial complex. Thus, the aim of this study was to morphometrically investigate the craniofacial complex of beta thalassemia subjects and compare it to matched healthy controls.

Material and Methods

Materials

This study was approved by the Ethics and Research Committee (School of Dentistry, National and Kapodistrian University of Athens, Greece).

The patients of the betathalassemia group (BTG) were in treatment in the Department of Hematology of Agia Sofia's hospital and referred to the Department of Oral Diagnosis & Radiology of the National and Kapodistrian University of Athens for dental screening and orthodontic consultation. Patients who met the following inclusion criteria were eligible to participate in the study: a) diagnosed with beta thalassemia, b) Caucasian origin, c) without previous orthodontic treatment and d) lateral cephalometric radiograph with a reference ruler

available. This BTG consisted of 40 patients ,24 males, 16 females, mean age 33.4(range 8.0 to 59.4 years).

A control group (CG) was selected from the patients seeking orthodontic treatment at the Department of Orthodontics, School of Dentistry, National and Kapodistrian University of Athens. The CG consisted of 80 healthy individuals, 48 males and 32 females, mean age 33.1 (range 7.9 to 60.4 years), who met the following inclusion criteria: a) Caucasian origin, b) no craniofacial malformations and syndromes, c) no previous orthodontic treatment, d) lateral cephalometric radiograph with a reference ruler available,. Each patient of the BTG was age and sex matched with two individuals from the CG.

Methods

The lateral cephalometric radiographs of both groups were scanned at a resolution of 150 dpi (Epson 1600 scanner, Seiko Epson Corporation, Nagano, Japan) and digitized using the Viewbox 4 software (dHAL software, Kifissia, Greece) by the same person (PR).

All radiographs were scaled to true size by the software, using the reference ruler. A total of 15 digitized curves described the main craniofacial structures. Their shape was represented by 127 landmarks that were distributed along the curves (Figure 1). Eleven landmarks were considered fixed and 116 were semilandmarks allowed to slide from their initial position along a vector tangent to their respective curve.

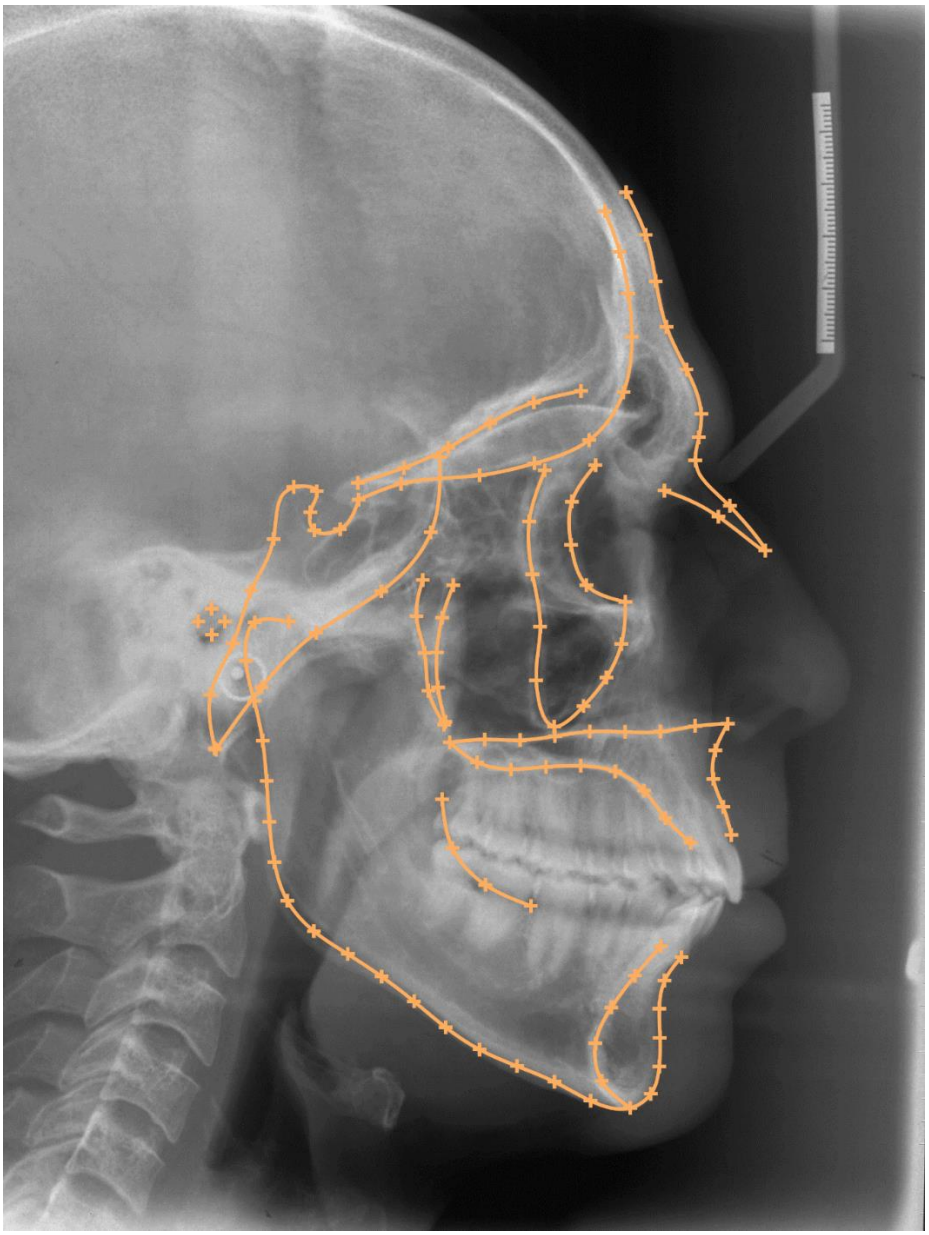

Figure 1. Description of the craniofacial complex with15 curves consisted of 127 landmarks (11 fixed and 116 semilandmarks). The areas traced included Po, Ba-S, endocranial surface of the frontal bone, maxilla and mandible, external and internal cortical plate of the frontal bone, orbit curve, zygomaticomaxillary curve, sphenoethmoidale, pterygomaxillary fissure, and ramus curve.

Sliding of the semilandmarks was performed against the average shape of the sample in the direction of minimizing the bending energy[14,15] The process was repeated three times and the average shape was recomputed after each iteration.

The final coordinates of the whole sample were subjected to generalized Procrustes analysis and principal component analysis (PCA).

Statistical analysis

In order to compare the groups, permutation tests (10,000 permutations without replacement) were conducted based on Procrustes distances between group means. Thalassemia and control groups were compared separately by sex. Comparisons were conducted for the craniofacial complex as a whole, as well as locally for the cranial base area, the midface and the mandible.

Error Estimation

Both intra- and inter-observer errors were calculated. Regarding intra-observer error, 20 lateral cephalometric radiographs were randomly selected from both groups one month after the first tracing and were re-traced with the same method.

For the assessment of inter-observer error, 20 lateral cephalometric radiographs were selected with the same randomised method and traced by another observer with previous experience in tracing cephalometric radiographs for morphometric studies.

Results

The first 4 principal components (PC1, PC2, PC3, PC4) accounted for 49.9% of the total sample's variability. PC1 and PC2 described variability in the vertical and anteroposterior direction, respectively. PC3 described the protrusion of the maxilla and the symphysis and PC4 local

changes in the gonial angle of the mandible (Figure 2, 3, 4). Approximately 85% of the total sample's variability was described by the first 20 PCs. (Table 1)

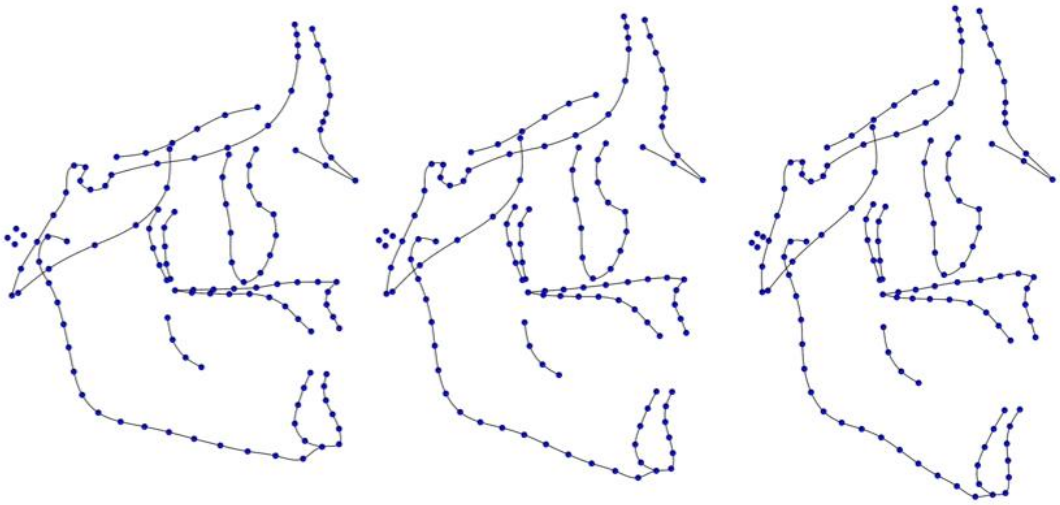

Figure 2. Shape variability of the craniofacial complex. Average shape is shown in the center and extremes along the PC1 direction (+/- 3 standard deviations) on the left and right..

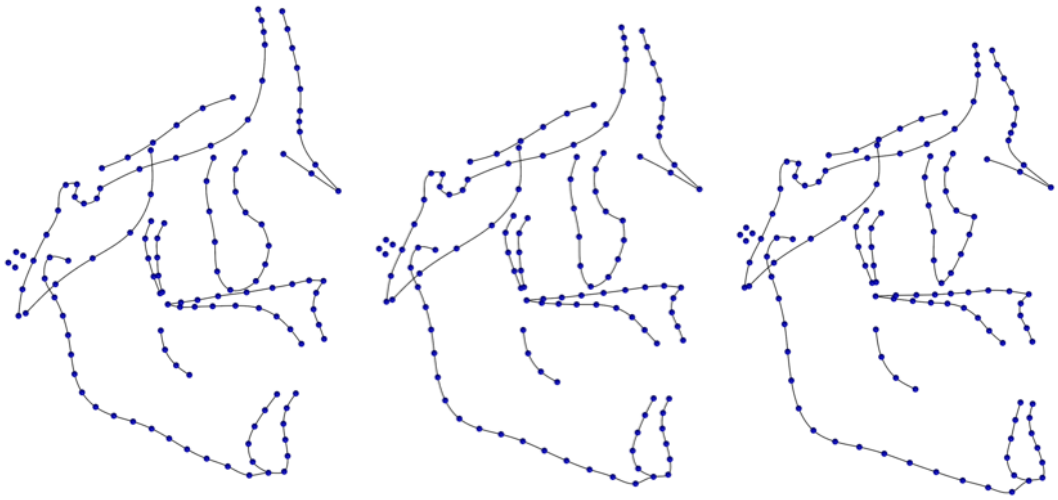

Figure 3. Shape variability of the craniofacial complex. . Average shape is shown in the center and extremes along the PC2 direction (+/- 3 standard deviations) on the left and right.

Statistical analysis was performed for each sex separately. PCA revealed that the major differences of the craniofacial complex were described in the male group by PC1, whereas PC2 mostly described the differences in the female group (Figure 5).

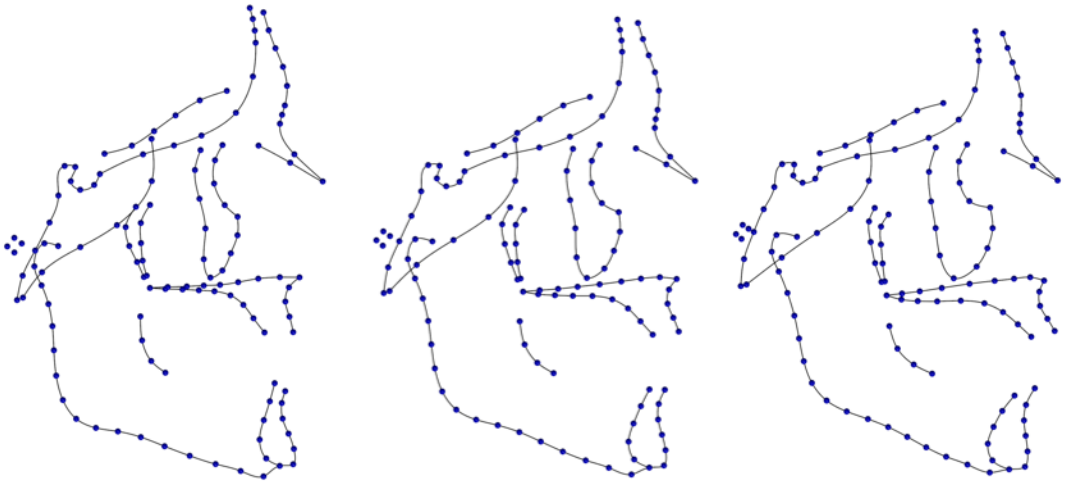

Figure 4. Shape variability of the craniofacial complex. Average shape is shown in the center and extremes along the PC3 direction (+/- 3 standard deviations) on the left and right.

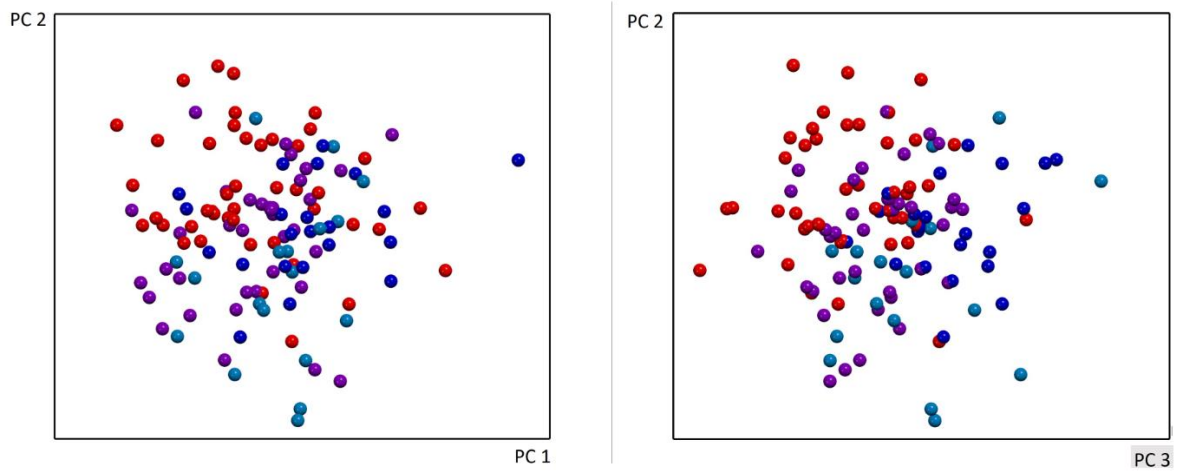

Figure 5. PCA plot for PC 1- PC 2, PC 2- PC 3. (Red: CG males, violet: CG females, blue: BTG males, light blue: BTG females)

Statistically significant differences in the morphology of craniofacial complex were detected between the two groups for males and females separately (P=0.000, P=0.015 respectively).

Permutation tests resulted in similar findings for the midfacial area (P=0.000 for males, P=0.029 for females), the mandible (P=0.000 for males, P=0.015 for females) and the cranial base (P=0.001 for males, P=0.016 for females). The average shapes of each male and female subgroup were superimposed to the matched control subgroups (figure 6). Superimposition of the average shapes of both groups showed a clear shape difference between the means, although differences in males were more pronounced in comparison to the differences in females. Regarding the structures near the cranial base, an increase was observed in vertical distance between the cranial base and the remaining facial structures. Both the endocranial surface and the internal cortical plate of the frontal bone were located in a superior position (figure 6, 7). The posterior cranial base and sphenoethmoidale structures were located with a more steep orientation. The zygomaticomaxillary area was located in a protruded position, especially in the male group, resulting in midface projection. The maxilla showed a small counter-clockwise rotation with increased palate thickness at its anterior and its posterior parts. The pterygomaxillary fissure had a more protruded position as well (figure 6, 8). At the mandibular area, patients with beta thalassemia showed a smaller mandibular body and a posteriorly positioned mandible in comparison with healthy subjects (figure 6, 9). Increased inclination of the mandibular body produced an increased gonial angle and a steeper mandibular plane. Combination of the above leads to a more convex and divergent profile in BTG.

Intra- and inter-observer errors were estimated as the mean Procrustes distance between the repeated digitizations and were 9.6% and 10.9% of the total sample's variance for intra-observer and inter-observer estimations, respectively.

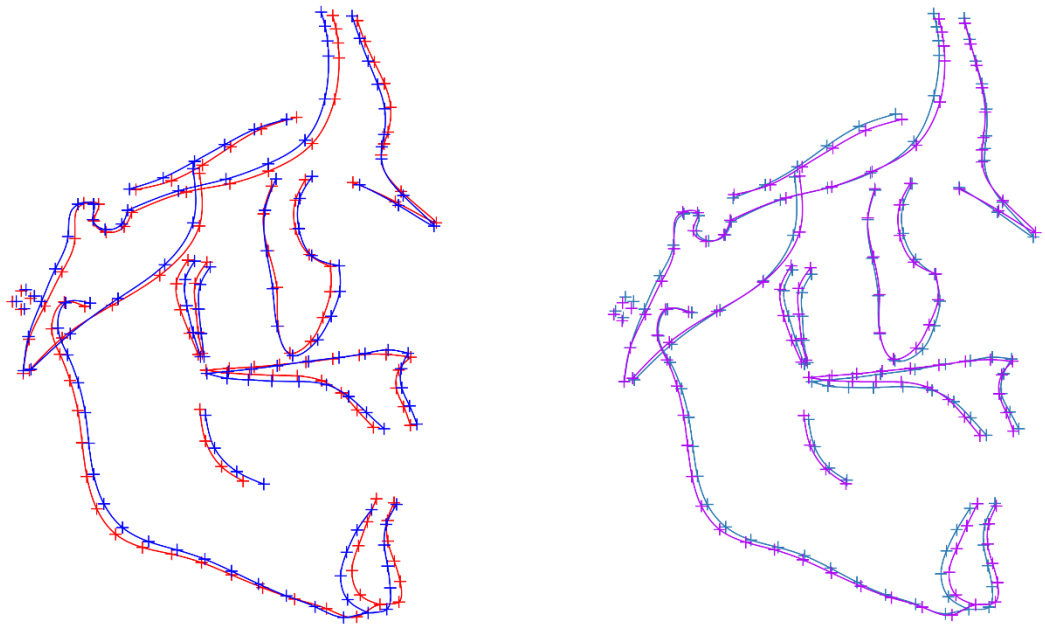

Figure 6. Superimposition of the average shapes of the craniofacial structures of males (left) and females (right) for BTG (dark blue males, light blue females) and CG (red males, purple females)

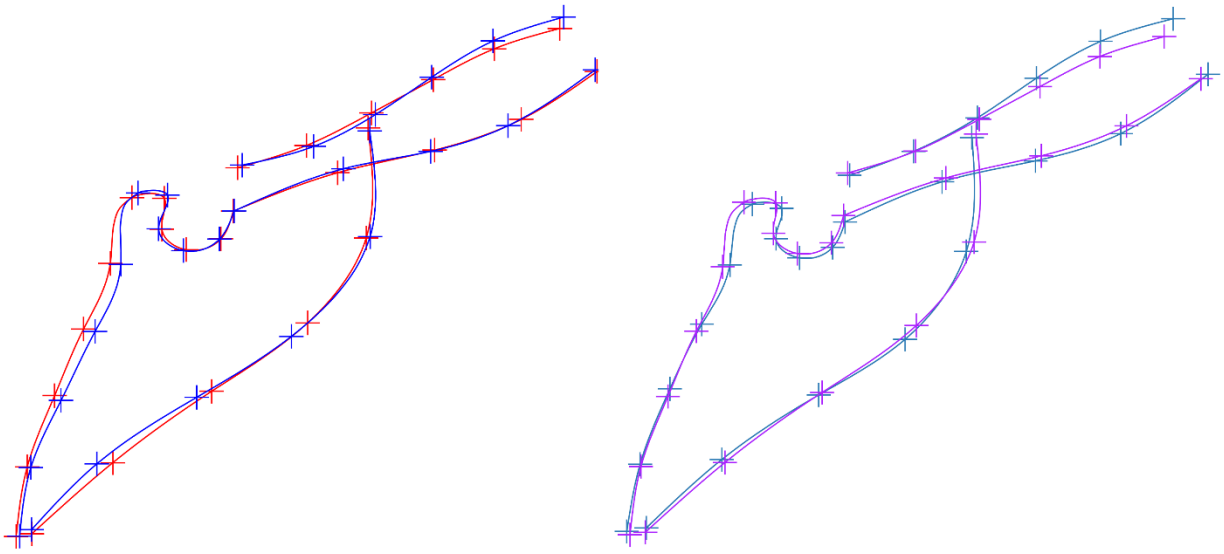

Figure 7. Superimposition of the average shapes of the cranial base of males (right) and females (left) for BTG (dark blue males, light blue females) and CG (red males, purple females)

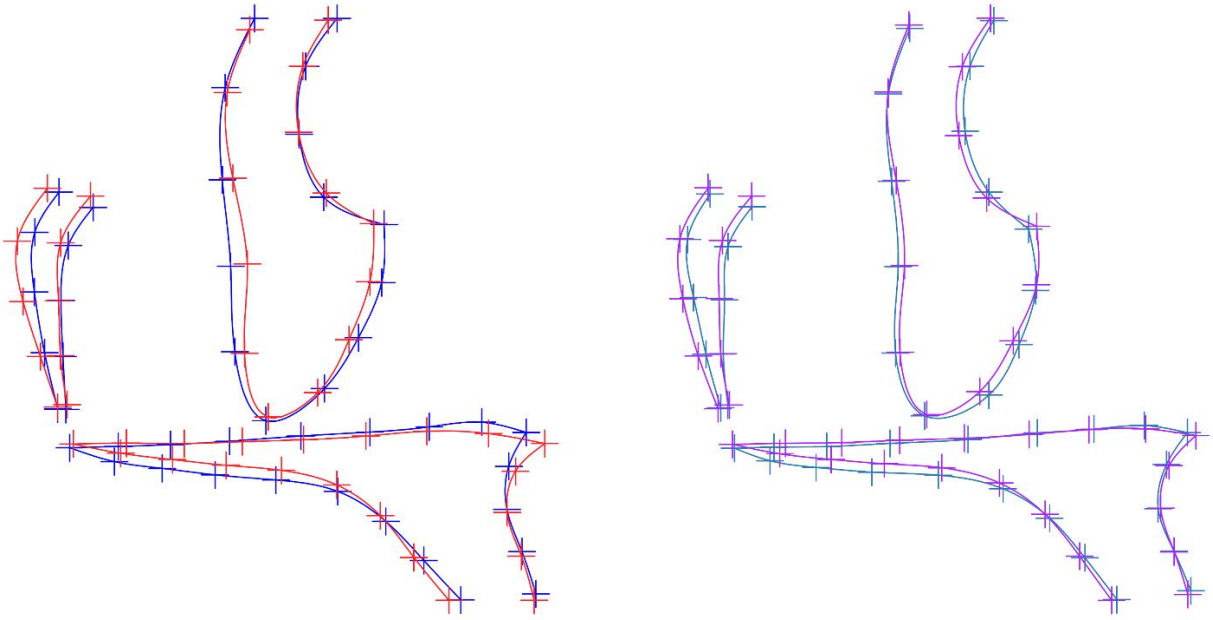

Figure 8. Superimposition of the average shapes of the midface of males (right) and females (left) for BTG (dark blue males, light blue females) and CG (red males, purple females)

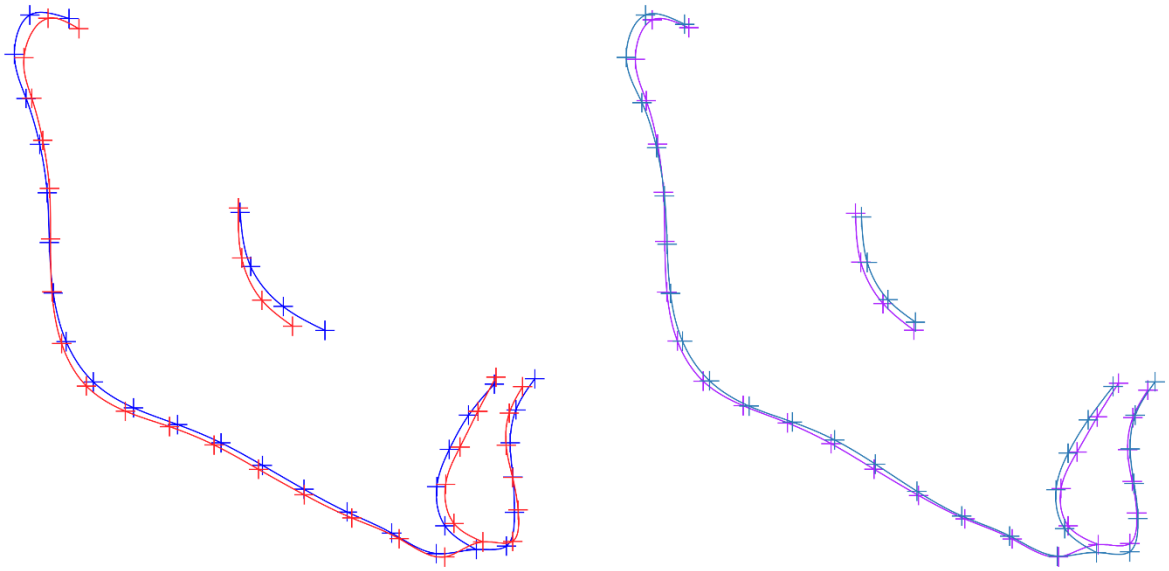

Figure 9. Superimposition of the average shapes of the mandible of males (right) and females (left) for BTG (dark blue males, light blue females) and CG (red males, purple females)

Table 1 Sample variability for the first 20 PCs

	%variance	% cumulative variance
PC 1	21.5%	21.5%
PC 2	14.3%	35.8%
PC 3	7.4%	43.2%
PC 4	6.7%	49.9%
PC 5	5.6%	55.4%
PC 6	4.0%	59.5%
PC 7	3.3%	62.7%
PC 8	3.0%	65.7%
PC 9	2.5%	68.2%
PC 10	2.2%	70.4%
PC 11	2.0%	72.4%
PC 12	1.9%	74.3%
PC 13	1.8%	76.1%
PC 14	1.7%	77.7%
PC 15	1.5%	79.3%
PC 16	1.4%	80.7%
PC 17	1.2%	81.9%
PC 18	1.1%	83.1%
PC 19	1.0%	84.1%

PC 20	1.0%	85.1%

Discussion

Beta thalassemia is well studied in a variety of medical fields. Haematologists and orthopaedists are the specialists mostly involved regarding quality of life, but dental professionals also contribute decisively in the management of beta thalassemia patients. Correction of their malocclusion can be achieved either with orthodontic or combined orthodontic-surgical treatment[16, 17]. The orthodontic-surgical approach may be more indicated, since beta thalassemia affects the growth of the craniofacial complex. However, overbleeding, stability problems due to multiple segmental osteotomies and the need to keep hemoglobin levels over 10mg/dl postoperatively are considerations that make this approach not suitable for all patients with beta thalassemia.[17]

Initial observational studies gave the term ''mongoloid'' to beta thalassemia patients' facial appearance.[18, 19] Those studies showed that thalassemia patients are more prone to a Class II skeletal pattern, without being able to define the exact structural differences from the normal population that could support their findings. Further observational anthropometric studies are available[20, 21, 22, 23, 24], however in the orthodontic literature only 5 studies could be found that used conventional cephalometrics to compare beta thalassemia patients with controls[5, 25, 26, 27, 28]. In this study we used a geometric morphometric approach on a large sample. The results agree with both the previous observational and cephalometric studies. Additionally, they

expand the current view providing a quantification of beta thalassemia manifestations and introduce a more detailed approach by considering sex subgroups.

Toman et al. (2011)[5]concluded that the vertical dimension is the most affected in thalassemia patients. They demonstrated an increase in the angles related with mandibular plane inclination and a reduction in posterior face height. They also reported a shorter mandibular body and ramus, but no differences in maxillary length. These findings agree with the results of the present study. Moreover, the present morphometric approach presented a reduction of the posterior to anterior facial height ratio. The counter clockwise rotation of the maxilla contributed to this height discrepancy too.

Amini et al. (2007)[25] agree with the results of the Toman et al. (2011)[5] study regarding maxillary length and position in relation to the cranial base, as well as regarding the smaller and retruded mandible. Vertical differences with reduction in posterior face height and a vertical face pattern were additionally reported. Concerning the cranial base, Amini et al. (2007)[25] did not find any differences. However, the GM approach revealed that the cranial base, the endocranial surface and the internal cortical plate of the frontal bone were superiorly positioned in BTG compared to CG in both sexes.

Abu Alhaija et al.(2002)[26] radiographically and clinically examined patients with beta thalassaemia major and divided the sample in three categories according to patients' dental age. Since the differences between sexes in their study were not statistically significant, male and female measurements in each dental age group were pooled. The authors used both conventional and the Wylie analysis in order to overcome the potential error of using the

Nasion point, since beta thalassemia patients may present with depression of the nose bridge. They reported a Class II pattern because of the relative projection of the maxilla on a shorter mandible and cranial base without augmentation in maxillary length. Superimposition of the two groups in the present investigation did not find a great amount of relocation of Nasion nor any differences in the length of the cranial base; however it highlights the relative projection of the maxilla. Both studies agree on a hyperdivergent profile with reduced posterior facial height.

Akkurt et al. (2017)[28] evaluated lateral cephalometric radiographs obtained from CBCT images of beta thalassemia patients. They found a high-angle Class II skeletal pattern, as indicated by the corresponding angular measurements (Sn/GoMe, ANS-PNS/GoMe, ANB, SNB, SNPog). Linear measurements also indicated an increased length of the maxilla and no statistical differences in length of the cranial base. They also investigated dental and soft tissue variables of the beta thalassemia sample and found protrusive lower incisors, retrusive upper incisors and an increased nasolabial angle.

As far as sexual dimorphism is concerned, conventional cephalometric studies have shown that men have increased total facial height and mandibular size, regarding both sagittal and vertical dimensions[29] (Miyajima 1996), yet the mandible is located in a clockwise rotated position in women. No sexual dimorphism in maxillary prognathism or inclination has been reported.[30] (Johannsdottir 2004). Geometric morphometric analysis has shown adequate power for assessment of sexual dimorphism[31, 32]. The present study agrees with the results emerged through both methodological approaches. Moreover, the present morphometric approach provided additional information on sexual differences, in that the zygomatic area was less protruded, whereas the frontal area was more protruded in males compared to females.

Conclusions

The anterior cranial base structures of beta thalassemia patients were located more superiorly and the posterior cranial base more posteriorly.

The midface was protruded with a counter-clockwise rotation of the maxilla. The mandible was smaller, posteriorly positioned and showed an increased gonial angle and clockwise rotation, resulting in a relative reduction of the posterior facial height.

The shape of the craniofacial complex in these patients is prone to be more convex and divergent.

Morphological differences tend to be more pronounced in male than in female beta thalassemia patients

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