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**Discriminant analysis of mandibular measurements for estimation of sex in a modern Brazilian sample**

**Concise / informative title: Mandibular measurements for estimation of sex**

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## **ABSTRACT**

The present study aimed to evaluate the accuracy of mandibular measurements for sex diagnoses in a Brazilian population. The sample was composed of 100 mandibles, of which 53 were female and 47 were male, with an average age of 57.03 years. The mandible measurement protocol was composed of 15 measurements, of which six were bilateral and nine were unique. Mandibles were directly measured using a digital caliper and a protractor. The descriptive analysis of the present study revealed higher mean values for male mandibles compared to female mandibles, except for the left mandibular angle. Among the 21 measures analyzed in this group, 15 were statistically significant ( $p < 0.05$ ). The univariate discriminant analysis showed a mean percentage of correct prediction varying between 49-79%. The association of variables increased the percentage of correct prediction of sex, varying between 76-86%. The ROC curve analysis indicated that the best variable for estimating sex was bigonial breadth (BGB) (AUC=0.764), followed by the right maximum ramus height (MRHd) (AUC=0.763). A reference table for estimating sex in a Brazilian population using mandible measurements was developed based on the ROC curve analysis. Mandibular measures provide a simple and reliable method for sex discrimination in Brazilian adults due to the sexual dimorphism shown by the analysis of the metric variables and the satisfactory results demonstrated by discriminant formulas, ROC curve analysis and the reference table.

**KEYWORDS:** Forensic Anthropology; Human Identification; Sex Determination by Skeleton; Forensic Dentistry; Mandible.

Sex estimation is an integral and fundamental step in biological profile construction due to the importance of this parameter in estimating other parameters, such as age, ancestry and stature [1]. In forensic anthropology, sex refers to the characterization of an individual by their reproductive system and secondary sex features in a biological approach, and this parameter has been shown to exhibit both inter- and intra-population variability [2]. Due to secular changes, males have developed greater and stronger muscularity compared to females, which results in larger bones and more robust and demarcated cranial traits [3,1].

The diagnosis of sex is not challenging for the examiner if a complete skeleton is available. Both the pelvis [4,5] and the cranium [6-8] provide highly accurate information. On the other hand, the analysis of sexual dimorphism in an incomplete or fractured skeleton can be a difficult task even for an expert. In addition to the pelvis and the cranium, the mandible is also considered a useful structure for providing information about the sex of an unknown skull. The use of mandibular measures has been studied since the beginning of the 20<sup>th</sup> century [9-15].

The interactions of the body with fauna and flora during the human decomposition process are of paramount importance regarding the changes that occur to the skeleton, and therefore, analysis of the context as a whole is essential in forensic anthropology [16,17]. Considering that the first joint to be disarticulated during the decomposition process is the temporomandibular joint, the mandible in most cases is the first anatomical structure found, or in some cases, it is not available for analysis due to the great variation in environments [18].

The mandible is considered an integral anatomical structure of the human cranium; it is a U-shaped bone and the only mobile bone of the skull. It is composed of two hemimandibles joined at the midline by a vertical symphysis. Each hemimandible is composed of a horizontal body with a posterior vertical extension termed the ramus [19]. The sexual dimorphism of mandibles is well known [14,11,20-22]. Qualitative methodologies have been described in the scientific literature, but due to the subjectivity of this type of methodology [23], metric analysis [21,14,15] and geometric morphometrics [24-26] are becoming more commonly used to overcome the disadvantages of morphological methodologies.

Discriminant analysis to develop discriminant functions to estimate sex is widely used in forensic anthropology and has yielded excellent results [27-33]. However, the discriminant functions present a population sensibility, and these functions should be developed and validated in different populations due to variations in ethnic parameters, which are directly related to phenotypic aspects [30,6].

The heterogeneous profile of the Brazilian population due to extensive migration justifies the importance of developing specific discriminant functions, as equations from other countries may not be valid for the national population. Considering Brazilian heterogeneity, the present study aimed to evaluate the accuracy of mandibular measurements for sex diagnoses in a Brazilian sample.

## **MATERIALS AND METHODS**

This investigation is in accordance with the international and national parameters of ethical investigations of human beings; the investigation protocol

was submitted and approved by the Ethics Committee of the University of São Paulo's School of Dentistry (FOUSP), process number 1.556.080.

The Brazilian sample was composed of 100 mandibles, of which 53 were female and 47 were male (aged between 18 to 104 years, with an average of 57.03), from the documented collection of the Institute of Teaching and Research in Forensic Sciences (IEPCF). The mandibles were exhumed from the Necrópolis Campo Santo cemetery located in the city of Guarulhos-Brazil. This is a documented collection, once all the biological profile information, such as sex, age, statute and ancestry were obtained from the public cemetery records.

The mandible measurement protocol was composed of 15 measurements, of which six were bilateral and nine were unique (Table 1). The linear measurements were taken in millimeters (mm) and the angular measurements in degrees (°) with two decimal digits. For analysis of the mandibles, the mandibular plane and the median sagittal plane were adopted as fundamental planes for protocol standardization.

[Table 1 here]

The mandibles were positioned using a mandible stabilizer. The device was developed to stabilize a mandible with the mandibular plane aligned in relation to the ground [34]. The equipment has a base, a fixation, a positioning table, and a measurement table. The patent registration was requested from the National Institute of Industrial Property in Brazil (INPI), BR 10 2013 003270-0 (Fig. 1).

[Figure 1 here]

After the correct fixation of the mandible and alignment of the fundamental planes described above, the measurement protocol was applied. Measurements of chin height (CHH), body height at the mental foramen (HMB), body thickness at the mental foramen (BMB), bimentale length (BML), bicoronoid breadth (BCB), bicondylar breadth (CBD), mandibular notch breadth (MNB), maximum ramus height (MRH), maximum mandibular length (MLT), bigonial breadth (BGB), mandibular length (projection) (MLP) and mandibular notch depth (MND) were made with a digital caliper (Lee Tools, Houston, Texas, USA) with an error margin of 0.01 mm. Conventional measurements were made, i.e., the fixed tip of the caliper was fixed to one of the reference points, and the movable tip was slid to the second point, measurements on the digital display of the device were read, and the values were recorded on a spreadsheet.

The minimum ramus width (MRB) and maximum ramus width (MARB) were measured using the measuring module coupled to the mandible stabilizer. This module comprises two measuring cars, two probe tips, a vertical spindle coupled to the vertical threaded spindle adjustment wheel, and a digital display. Measurements are made by opening the two measuring carriages, causing the probe tips to be opened accordingly. The two tips are positioned to palpate the anterior margin and posterior margin of the mandible ramus. Then, through vertical spindle movement, the probe tips move horizontally through the mandibular ramus, and through horizontal spindle movement, the perpendicularity of the mandibular branch in relation to the ground is corrected. This procedure is carried out by observing the reading on the digital display of the measuring module until the lowest (MRB) and highest values (MARB) are



found. Only two measurements were performed on the left side due to the fixation of the measurement module (Fig. 2).

[Figure 2 here]

The mandibular angle (MA) was obtained using a 360° protractor. An aluminum bar with a thickness of 1 mm was attached perpendicular to the protractor to standardize measurements. The bar tangency, the posterior margin of the mandibular ramus, and the base of the stabilizer served as support for the lower margin of the mandible and for the protractor, allowing the measurement to be made (Fig. 3).

[Figure 3 here]

The protocol was applied by two observers to verify reproducibility and repeatability of the methodology. The inter-observer and intra-observer concordance tests were performed using the intraclass correlation coefficient (ICC).

The data were tabulated using Microsoft Excel. Afterwards, a descriptive statistical analysis was applied using a t-test. Univariate and multivariate discriminant analysis were used to study the sexual dimorphism of the sample. The discriminant function was created for each variable analyzed for each sex and was built as follows:  $P = a + b_1 \cdot x_1 + b_2 \cdot x_2 + \dots + b_m \cdot x_m$ , where  $a$  is a constant,  $b_1$  through  $b_m$  are the discriminating coefficients, and  $x_1$  through  $x_m$  are the discriminating variables. To estimate the sex, the values of the measurements need to be included in the equation for males and females so that the greatest product of  $P$  indicates the sex. In addition, the sexual bias of the classification accuracy was described as the difference between the male

classification accuracy and female classification accuracy, where greater sexual bias indicates greater differences between the two sexes.

Receiver operator characteristic (ROC) curve analysis was applied to the two samples to analyze the sexual dimorphism and to develop a reference table for the Brazilian population using mandibular parameters. The percentages of correct classification considered for constructing the table were 75%, 80%, 85% and 90%, and the demarking points were calculated using the mean of the medium values of the sexes [7]. All tests were performed at a significance level of 5%, and all statistical analyses were carried out using the statistical programs MedCalc, Minitab and Stata 14.2.

## RESULTS

The inter- and intra-observer analysis showed that all variables presented excellent correlation, with an intraclass correlation coefficient greater than 0.75.

The mandibular measurements are described in Table 2. Only the left mandibular angle (MAe) presented a higher mean for the female sex; the other variables presented higher averages for the male sex. Among the 21 measures analyzed in this group, 15 presented a statistically significant result: right (HMBd) and left (HMBe) body height, bimentale length (BML), bicoronoid breadth (BCB), bicondyliar breadth (MRB), right (MRHd) and left (MRHe) maximum ramus height, maximum mandibular length (MLT), bigonial breadth (BGB), mandibular length (MLP), and right (MNDd) and left (MNDe) mandibular notch depth. Among these, the measures that presented the highest sexual dimorphism were bigonial breadth (BGB), right (MRHd) and left (MRHe) maximum ramus height, mandibular length (MLP) and bicondyliar breadth

(CDB). Using these measures, the difference between the sexes could be explained in 16.92 to 21.11% of the cases.

[Table 2 here]

The univariate discriminant analysis showed a mean percentage of correct prediction varying from 49-79%. The bigonial breadth (BGB) showed the highest accuracy (79%), and the left body thickness at mental foramen (BMBe) showed the lowest (49%) (Table 3). In the univariate discriminant analysis of the mandibular measurements, only the bigonial breadth (BGB) corresponded to an average percentage above 75%; therefore, the multivariate analysis was performed using the variables that presented a correct prediction percentage above 65%. Table 4 shows the functions and their respective percentages of correctness, with a variation of 76-83%.

[Table 3 here]

[Table 4 here]

Analysis of the area under the ROC curve (AUC) showed that, except for the bicoronoid breadth (BCB) (0.682), all variables can correctly determine sex with an accuracy of greater than 70%. The cut-off points presented in the table represent the ideal points for sex discrimination for each variable, with the percentages of correctness for each sex regarding each measure (Table 5). A reference table for the Brazilian population using mandibular measurements was developed based on the results of the ROC curve analysis (Table 6).

[Table 5 here]

[Table 6 here]

## DISCUSSION

The construction of a biological profile is the main objective of forensic anthropology and an important step in improving possible positive identification, thus helping to reduce the number of individuals. Sex estimation is one of the most important elements in the creation of this profile, as the estimation of the other aspects is indirectly linked to accurate sex estimation [1]. The heterogeneous Brazilian ethnographic profile is an important aspect that must be considered in forensic cases. Methodologies and functions developed for other nationalities do not correspond to the same accuracy in this specific population, highlighting the importance of implementing national parameters.

The descriptive analysis of the present study revealed higher mean values for male mandibles compared to female mandibles, except for the left mandibular angle [14,20-22]. Among the variables analyzed, 71.42% presented sexual dimorphism; the bigonial breadth presented the highest sexual dimorphism, followed by the right (MRHd) and left (MRHe) maximum ramus heights, mandibular length (MLP) and bicondylar breadth (CDB). İlgüy et al. [20] showed that the gonial angle, ramus length, mandibular base length and bigonial breadth were reliable for analyzing sexual dimorphism. Dong et al. [11] demonstrated that the most dimorphic measurements for mandibles were the maximum mandibular length and the bicondylar breadth.

The univariate discriminant analysis varied between 49-79%, and the bigonial breadth showed the highest accuracy. The association of the variables increased the percentage of correct sex predictions, which varied between 76-

86%. The accuracy rates found in the present study are in agreement with recent literature [14,31,24,20,22]. On the other hand, these results revealed an error estimation ranging from 17% to 24%, which should be considered in the identification process. Besides that, these results highlight the importance of using several methodologies to construct the biological profile in forensic anthropology cases. Carvalho et al. [14] performed a study with 66 Brazilian mandibles (34 male and 32 female) using two linear measurements (bigonial breadth and the mandibular ramus height). The results showed a percentage of correctness of 78.13% for females and 76.47% for males. The authors concluded that the method involving physical anthropology was highly accurate for human identification and could be easily applied at low cost.

Future studies are required to validate the present mandibular functions due to the small sample size of the present study. The use of imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) to validate methodologies has increased due to the lack of large bone collections combined with the ease of accessing CT or MRI images [1,15]. İlgüy et al. [20] analyzed 161 three-dimensional images of adult patients, 66 males and 65 females. Cross-sectional analysis using the foramen magnum and mandible measurements revealed an average accuracy of approximately 83.2% (77.3% for females and 87.4% for males). The authors concluded that the gonial angle, ramus length, mandibular base length, bigonial breadth of the mandible and the foramen magnum length were parameters that could aid in the analysis of sexual dimorphism using computed tomography. Dong et al. [11] concluded that the analysis of CT scans could be used as an alternative resource for the application of osteometric techniques.

The ROC curve analysis demonstrated that the results in the present study are in agreement with previously obtained results. The best variable for estimating sex was the bigonial breadth (BGB) (AUC=0.764), followed by the right maximum ramus height (MRHd) (AUC=0.763). The development of population-specific tables that optimize anthropological examinations and, at the same time, ensure satisfactory accuracy is extremely relevant to forensic sciences. The present study, through ROC curve analysis, developed a mandibular measurements reference table for Brazilian individuals. Future studies are necessary to validate this table and confirm the results obtained in the present study through the statistical analysis performed.

A standard mandibulometer was not used because it wasn't available. Few laboratories in Brazil have mandibulometers so we aimed to simplify the methodology of measure the gonial angle, so it could be applied easily in the Brazilian Identification services. Also, the mandibular stabilizer was developed to stabilize the mandible with the mandibular plane aligned in relation to the ground, once all other measures from the protocol were acquired using the mandibular stabilizer, it was decided to gauge the gonial angle using the equipment described.

The various methodologies used to date have shown the importance of forensic anthropology for the delineation of biological profiles, especially the estimation of sex of an unknown skeleton. The importance of studying different populations is also clear, as regional variations and interaction with the environment have major impacts on the phenotypic characteristics of the individual.

## **CONCLUSION**

In conclusion, mandible variables are important parameters for estimating sex in the Brazilian population. The reference table and the discriminant functions provided a simple and reliable method for sex discrimination of Brazilian adults.

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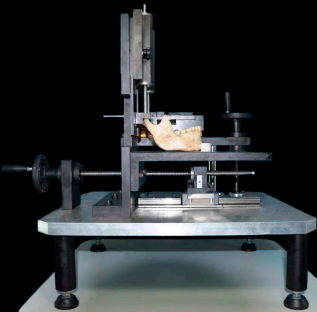
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Table 1. Mandibular measurement protocol.

Measure	Definition
Chin height (CHH)	Linear distance from <i>infradentale</i> (id) to <i>gnathion</i> (gn).
Body height at mental foramen (HMB)	Distance from the alveolar process to the inferior border of the mandible at the level of the mental foramen.
Body thickness at mental foramen (BMB)	Maximum breadth at the level of the mental foramen and perpendicular to the long axis of the mandibular body.
Bimentale length (BML)	Linear distance between right and left <i>mentale</i> (ml).
Bicoronoid breadth (BCB)	Distance between the highest points of the mandibular coronoid processes.
Bicondylar breadth (CDB)	Linear distance between right and left <i>condylion laterale</i> (cdl).
Mandibular notch breadth (MNB)	Distance between the superior point of the condylar process and the superior point of the coronoid process.
Minimum ramus breadth (MRB)	The minimum breadth of the mandibular ramus measured perpendicular to the height of the ramus.
Maximum ramus breadth (MARB)	The maximum breadth of the mandibular ramus measured perpendicular to the height of the ramus.
Maximum ramus height (MRH)	The distance from gonion (go) to the highest point on the mandibular condyle.
Maximum mandibular length (MLT)	The distance from the anterior margin of the chin to the midpoint of a straight line extending from right gonion (go) and left gonion (go).
Bigonial breadth (BGB)	Linear distance between right and left gonion (go).
Mandibular length (Projection) (MLP)	Distance between pogonion (pg) and the perpendicular line that tangent the posterior part of the condylar processes.
Mandibular angle (MA)	The angle formed by inferior border of the body and the posterior border of the ramus.
Mandibular notch depth (MND)	Distance between the inferior point of the mandibular notch and the midpoint of a straight line extending from the superior point of the condylar process and the superior point of the coronoid process.

Table 2. Descriptive statistics and comparison of mean for sexual dimorphism using mandibular measures.

Measure <sup>a</sup>	Male				Female				<i>t</i>	<i>p-value</i>
	Mean	SD	95 % CI		Mean	SD	95 % CI			
CHH	27.39	6.55	25.58	29.19	25.36	6.62	23.41	27.3	1.53	0.1273
HMBd	26.37	5.35	25.89	27.84	22.9	5.3	21.34	24.46	3.24	0.0016*
HMBe	26.57	5.61	25.03	28.12	22.62	5.02	21.14	24.09	3.69	0.0004*
BMBd	10.57	1.95	10.03	11.11	10.28	1.61	9.81	10.76	0.78	0.436
BMBe	10.29	2.15	9.69	10.88	10.29	1.7	9.79	10.79	0	0.9991
BML	44.8	2.88	44.01	45.6	43.5	2.96	42.63	44.37	2.23	0.0280*
BCB	95.63	6.28	93.9	97.36	91.96	4.75	90.56	93.35	3.26	0.0015*
CDB	117.08	6.49	115.21	118.95	112.07	4.42	110.74	113.4	4.32	0.0000*
MNBd	33.24	3.43	32.28	34.21	31.57	3.05	30.67	32.46	2.54	0.0126*
MNBe	33.6	3.91	32.5	34.7	31.59	2.81	30.75	32.42	2.87	0.0049*
MRB	30.46	3.91	29.37	31.55	28.94	3.26	27.98	29.9	2.08	0.0401*
MARB	32.46	3.78	31.41	33.5	31.02	3.65	29.95	32.1	1.92	0.0577
MRHd	60.57	6.26	58.82	62.31	54.73	5.24	53.19	56.27	4.99	0.0000*
MRHe	59.26	6.02	57.55	60.98	54.09	5.19	52.55	55.63	4.48	0.0000*
MLT	69.81	5.21	68.37	71.25	67.02	5.23	65.49	68.56	2.65	0.0092*
BGB	92.63	5.79	91.03	94.23	87.02	5.07	85.53	82.51	5.12	0.0000*
MPL	104.02	6.25	102.22	105.81	98.05	5.84	96.34	99.77	4.78	0.0000*
MAd	122.07	7.85	119.91	124.23	121.79	6.93	119.76	123.83	0.18	0.8526
MAe	122.09	8.82	119.66	124.52	122.22	6.73	120.24	124.2	-0.08	0.9353
MNDd	13.76	1.95	13.22	14.31	12.29	1.84	11.74	12.83	3.83	0.0002*
MNDe	13.87	1.89	13.32	14.42	12.35	2.24	11.67	13.02	3.52	0.0007*

SD= standard deviation/ IC= confidence interval

Table 3- Direct discriminant analysis of the mandibular measurements.

Measure	$\lambda$ Wilks	Correct Prediction % ♂	Correct Prediction % ♀	Mean Correct Prediction %	Sex bias
HMBd	0.903	71.70	51.10	62.00	20.6
HMBe	0.878	77.40	55.30	67.00	22.1
BMBd	0.994	47.20	66.00	56.00	-18.8
BMBe	1,000	41.50	57.40	49.00	-15.9
BML	0.952	71.70	46.80	60.00	24.9
BCB	0.902	60.40	70.20	65.00	-9.8
CDB	0.985	41.50	93.60	66.00	-52.1
MNBd	0.937	54.70	61.70	58.00	-7
MNBe	0.959	43.40	74.50	58.00	-31.1
MRB	0.957	54.70	70.20	62.00	-15.5
MARB	0.964	60.40	46.80	54.00	13.6
MRHd	0.795	67.90	72.30	70.00	-4.4
MRHe	0.83	56.60	78.70	67.00	-22.1
MLT	0.933	56.60	44.70	51.00	11.9
BGB	0.717	73.60	85.10	79,00*	-11.5
MLP	0.81	77.40	59.60	69.00	17.8
MAd	1,000	79.20	25.50	54.00	53.7
MAe	1,000	52.80	63.80	58.00	-11
MNDd	0.867	71.70	61.70	67.00	10
MNDe	0.909	35.80	76.60	55.00	-40.8

Table 4- Discriminant equations, group centroids and correct assignment by sex.

Function and Measure	Fisher Coefficient		Group centroid	Sectioning point	$\lambda$ Wilks	Correct prediction (%)	
HMBe	-0.844	-0.892					
BCB	0.761	0.777					
CDB	2.999	2.916					
MRHd	0.439	0.333					
MRHe	-0.74	-0.691					
BGB	0.793	0.674					
MLP	2.331	2.257					
MNDd	1.775	1.536					
Constant	-363.455	-329.690					
						Male= -363,455+ (-0,844*HMBe)+(0,761*BCB)+(2,999*CDB)+(0,439*MRHd)+(-0,740*MRHe)+(0,793*BGB)+(2,331*MLP)+(1,775*MNDd) Female= -329,690+ (-0,892*HMBe)+(0,434*BCB)+(0,573*CDB)+(0,638*MRHd)+(0,630*MRHe)+(0,674*BGB)+(2,257*MLP)+(1,536*MNDd)	
MLP	1.821	1.738					
BGB	2.444	2.293					
MRHd	1.067	0.944					
MRHe	-0.442	-0.43					
Constant	-223.570	-198.175					
						Male= -223,570+(1,821*MLP)+(2,444*BGB)+(1,067*MRHd)+(-0,442*MRHe) Female= -198,175+(1,738*MLP)+(2,293*BGB)+(0,944*MRHd)+(-0,430*MRHe)	
MLP	2.109	1.990					
BGB	2.445	2.294					
Constant	-223.570	-198.175					
						Male= -223.570+(2.445*BGB)+(2.109*MLP) Female= -198.175+(2.294*BGB)+(1.990*MLP)	



Table 5- ROC curve analysis of mandibular measurements.

Measure	AUC	<i>p-value</i>	Lower limit	Upper limit	Section point		Male (%)	Female (%)	
HMBe	0.725	<0,001	0.626	0.824	♀<	27.23	>♂	53.85	83.33
BCB	0.682	0.002	0.578	0.787	♀<	96.98	>♂	44.23	91.67
CDB	0.737	<0,001	0.635	0.839	♀<	116.11	>♂	62.5	84.78
MRHd	0.763	<0,001	0.669	0.857	♀<	56.56	>♂	76.9	70.2
MRHe	0.738	<0,001	0.639	0.837	♀<	56.6	>♂	76	67.4
BGB	0.764	<0,001	0.671	0.857	♀<	90.4	>♂	67.9	80.9
MLP	0.753	<0,001	0.658	0.848	♀<	101.72	>♂	75.5	70.2
MNDd	0.712	<0,001	0.61	0.814	♀<	13.11	>♂	68.63	68.09

Table 6- Reference table based on ROC curve analysis for sexing estimation by mandibular measurements in a Brazilian population.

Variable	Male				Determination Point	Female			
	95%	90%	80%	70%		70%	80%	90%	95%
HMBe	34.34	32.92	31.21	29.77	24.6	19.94	17.61	16.24	13.97
BCB	105.06	103.89	100.67	98.79	93.8	89.38	88	85.91	83.39
CDB	127.73	125.25	123.47	121.9	114.58	109.15	108.62	106.77	105.05
MRHd	71.53	69.75	66.28	64.21	57.65	52.59	50.87	48.16	46.83
MRHe	69.32	67.77	65.18	62.77	56.68	51.1	49.7	47.38	45.66
BGB	105.96	99.25	97.3	96.11	89.83	83.03	82.51	81.83	79.77
MLP	116.66	114.99	109.74	105.91	101.04	95.06	93.4	91.28	86.77
MNDd	17.19	16.27	15.74	14.78	13.03	11.79	10.72	9.7	9.08