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Menstrual disorders and associated factors among rural and tribal adolescent girls in India: A systematic review and meta-analysis

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ABSTRACT: After attaining menarche adolescents, due to shyness and fear, often refuse to seek medical treatment. Simultaneously they began to face menstrual disorders. The present review aimed to estimate the overall menstrual disorders and associated factors among adolescent girls in rural and tribal areas in India as well as to summarize the most recent research findings on the pooled prevalence of menstrual disorders. The study design was developed applying the PRISMA checklist-2020. The whole protocol was registered on PROSPERO (Registration ID: CRD42024385046). Articles (English language) related to menstrual irregularities among 10 to 19-year-old adolescent girls in India were collected based on inclusion and exclusion criteria from 2000 to 2023 followed by selected keywords. The quality assessment of the present study was evaluated using the CASP (Critical Appraisal Skills Programme) checklist. Meta-analysis was conducted by using MedCalc software version 22.0. Publication bias was checked using Egger's test. A total of 61 studies (47 from rural and 14 from tribal areas) in India have been evaluated. The random effect model showed an overall prevalence of dysmenorrhea, irregular menstruation, PMS, oligomenorrhea, polymenorrhea and menorrhagia in both areas was 54.96% (95% CI: 47.93 to 61.85), 26.21% (95% CI: 20.73 to 32.09), 47.49% (95% CI: 31.44 to 63.81), 13.88% (95% CI: 8.98 to 19.65),7.85% (95% CI: 2.30 to 16.31), 16.83% (95% CI: 10.04 to 24.96) respectively. Among these, dysmenorrhea, irregular menstruation, and PMS were found to be the most predominant in both areas. Lack of physical activities, dietary habits, BMI, socioeconomic factors, and socio-cultural taboos were found to have a strong association with menstrual irregularities. Prior and after attaining menarche, proper guidance on every aspect of menstruation should be urgently arranged in schools and at home to get rid of fear and anxiety, so that adolescent girls can cope with menstrual-related issues. Health camps should be organized in both areas to allow an easy access.

KEY words: menstrual disorders, associated factors, adolescent girls, rural, tribal, India, systematic review, meta-analysis

Original article

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Introduction

Menstruation is a health issue that has three dimensions: a physiological, psychological, and social process that needs to be addressed before menarche and after menopause (WHO 2022). Adolescent girls are often not aware of menarche hence the first period can be accompanied by fear, and anxiety along with stigma, taboos, and myths (UNICEF, 2018). Because of these myths and misconceptions about menstruation, girls often develop negative attitudes towards this pubertal development process (Walia et al. 2015). Being a secret and personal matter, mothers used to merely talked about this aspect of life hence friends were the sources of information about menarche (Dhingra et al. 2009). Dysmenorrhea or abdominal cramps, pre-menstrual syndromes (PMS), and irregular menstruation were the most common disorders among adolescent girls in rural areas of West Bengal (Sanyal and Ray 2008; Ray et al. 2010). More than 70% of tribal adolescent girls were experiencing dysmenorrhea (Nagar and Aimol 2010; Shanmugananth et al. 2023). By neglecting this issue, menstrual disorders have been increasing, especially among late adolescent girls (Singh and Kasturwar 2017; Sharma et al. 2019). Interestingly, a recent systematic review on menstrual hygiene practices and menstrual morbidities among adolescent girls in India showed that the most prevalent disorders were dysmenorrhea, PMS, oligomenorrhea, menorrhagia and polymenorrhea (Majeed et al. 2022). It has been reported that about 64% of adolescent girls experience at least one type of problem-related to menstruation (Pearlstein and Steiner 2008).

Nowadays there are lot of changes in the daily lifestyle of adolescents. Dietary habits and physical activity are the two major concerns that were significantly associated with disorders during menstruation (Negi et al. 2018). The daily routine was interrupted due to menstrual irregularities, resulting in prolonged bed rest, sleep disturbances along school absenteeism (Sharma et al. 2008). Nutritional status and socio-economic condition were associated with menstrual irregularities (Verma et al. 2021). The age of the respondents was also associated with menstrual-related problems (Sanyal and Ray, 2008; Nagar and Aimol 2010). Menstrual disorders also had a significant association with family history (Kumbhar et al. 2011).

Systematic reviews generally have a detailed search strategy by synthesizing all relevant articles on a particular topic to reduce publication bias. That feature makes a systematic review more reliable and different from a narrative review (Uman 2011). Systematic review is usually done to reduce random errors and bias. Sometimes systematic review comes with meta-analysis, which involves a statistical technique to create all quantitative data retrieved from all the studies into single or combined results to give a clear idea about the particular topic (Petticrew and Roberts 2008).

Although there many reviews have been published on Menstrual Hygiene Management, among urban, rural as well as tribal adolescent girls in India, no reviews, to our knowledge, have focused on menstrual disorders among rural and tribal adolescent girls in India till now. Hence this review is very important to identify the menstrual problems among rural and tribal India as well as associated factors during menstruation that can be linked with Sustainable Development Goals (SDGs), specifically, SDG 3 states "Good Health and Well Being".

Study objectives

1. To determine the prevalence of various physical disorders and associated factors during menstruation experienced by rural and tribal adolescent girls in India.

2. To discern the overall menstrual disorders among adolescent girls in rural and tribal areas in India as well as to summarize the most recent research findings on the pooled prevalence regarding this topic.

Methodology

Trial registration number

The whole protocol of the present review was registered on PROSPERO (Registration ID: CRD42024385046).

Study criteria

Data extraction and inclusion were done based on previously framed inclusion and exclusion criteria (Tab. 1).

Study design and search strategy

The study design was developed in line with the "preferred reporting items for systematic reviews and meta-analysis" (PRISMA) checklist (Page et al. 2021). Studies published in English language from the year 2000 to 2023 focusing on the prevalence of menstrual disorders among 10 to 19-year-old adolescent girls from rural and tribal areas in India were included. Data were collected from the following databases: PubMed Central, NCBI, Research Gate, Academia and Google Scholar, Science Direct, BMC, and PLOS ONE. Some keywords from MeSH have been used to make this review more reliable and combined through the Boolean operator ("AND", "OR") i.e, "menstrual disorders" OR "menstrual irregularities" OR "menstrual health status" OR "menstrual patterns" OR "menstrual disturbances", "dysmenorrhoea" OR "abdominal cramps" OR "pain in the abdomen", "menorrhagia" OR "heavy menstrual bleeding", "irregular menstruation" OR "irregular menses" OR "irregular cycle", "oligomenorrhea" AND "polymenorrhea", AND "amenorrhea", AND "hypomenorrhea", AND "pre-menstrual syndromes", AND "adolescent girls", AND "rural", AND/OR "tribal", "India", "cross-sectional" AND "Association" OR "predictors". PRISMA checklist for systematic reviews has been followed throughout this review, presented in a supplementary file.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
1. Articles were limited to India only.	1. Beyond India.
2. Studies related to 2000–2023.	2. Before 2000 and after 2023.
3. Targeted population aged between 10–19 years among rural and tribal areas only.	3. Adolescents aged less than 10 years and over 19 years or adult population or belonging to the urban areas.
4. Clearly defined community-based cross-sectional studies related to menstrual disorders.	4. Review papers, short articles, letters to editors, disorders vaguely mentioned articles.
5. Comparative study.	5. Case-control and intervention-based study.

Data collection and selection

An initial search of a total of 350 (abstract and full text) articles was carried out from databases such as PubMed Central, NCBI, Research Gate, Academia, Google Scholar, Science Direct, BMC, and PLOS ONE. Cross references were also considered in searching for relevant articles. Based on inclusion and exclusion criteria, a total of 289 articles have been extracted. After the final screening, a total of 61 articles (47 from rural, 14 from tribal areas, and 1 paper belonging to rural and tribal areas) among Indian adolescent girls have been included. A flow diagram of selecting articles is depicted in Figure 1. To construct keyword co-occurrences a networking map was created by using an online software tool "VOSviewer" version 1.6.20 (Fig. 2). The greatest number of co-occurrences of keywords were adolescents, menstruation, menstrual disturbances, and India (Fig. 2).

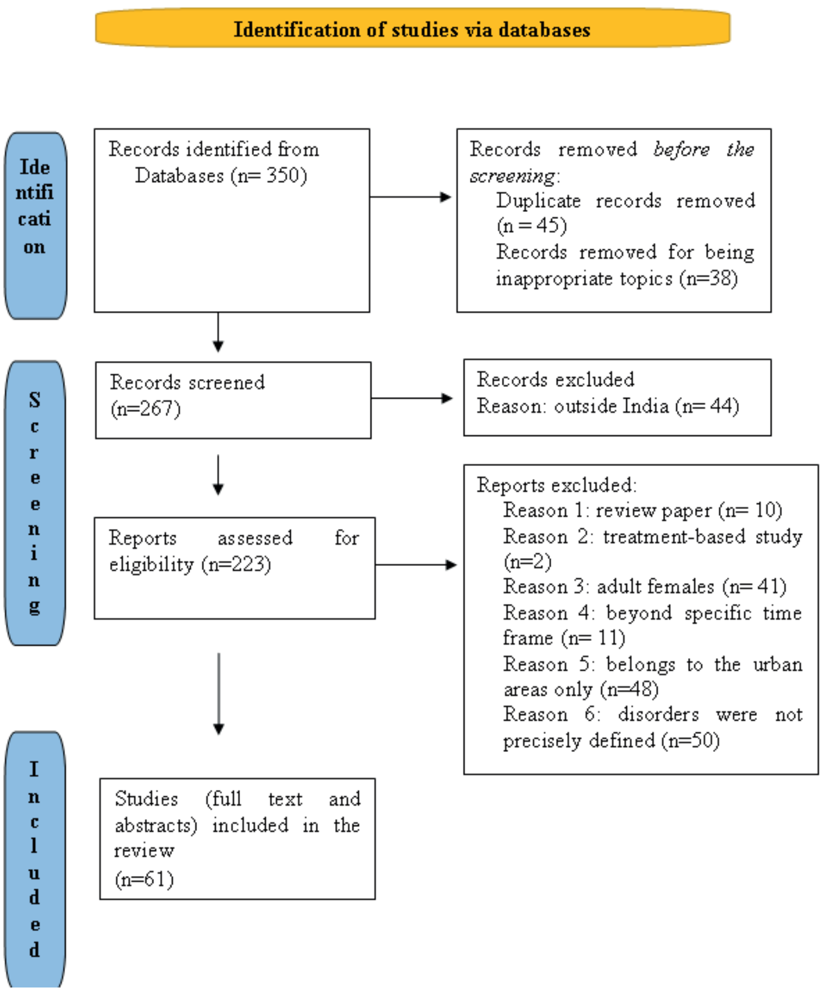

Figure 1. PRISMA flow diagram of the present systematic review

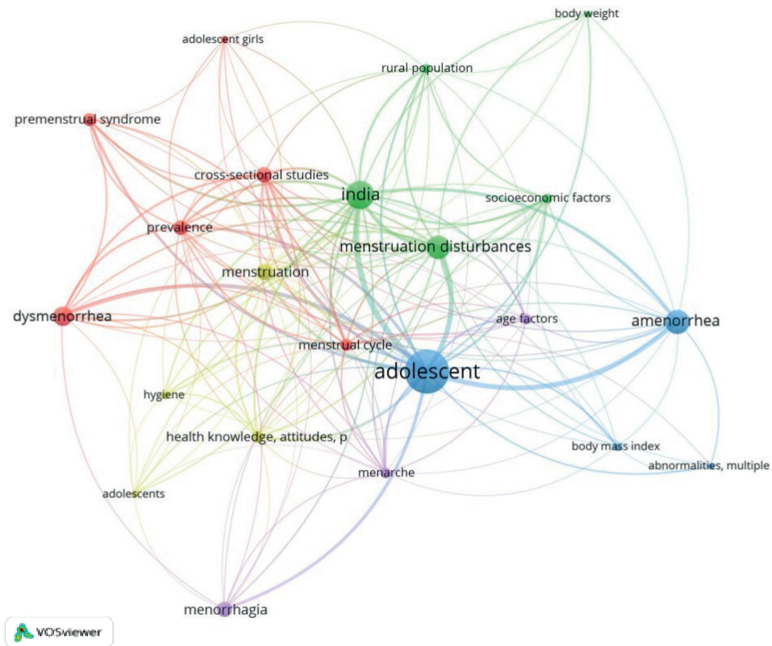

Figure 2. Keyword co-occurrences of included studies

Quality assessment of the study

The quality assessment was evaluated by the CASP (Critical Appraisal Skills Programme) systematic review checklist as shown in the supplementary file.

Ethical approval

All included studies were based on secondary data, hence ethical approval is not needed.

Study analysis

Meta-analysis was done by using MedCalc software version 22.0.

Test of heterogeneity

Heterogeneity within studies included in the meta-analysis was represented by a forest plot and publication bias in the present study was represented through a funnel plot or scatter plot. The test of heterogeneity was done by Cochrane's Q statistic-based I^2 statistic. Scores of heterogeneities are measured by three types, i.e., 25% (low), 50% (moderate), and 75% (high heterogeneity). The study is considered homogenous when $I^2<50\%$ and thus a fixed effect model is considered. P value<0.1 or I^2 value> 50% indicates heterogeneity and thus a random effect model (DerSimonian and Laird, 1986) should be used to reduce bias. Heterogeneity scores in this study were above 50%, hence we used the random effect model. Each study was represented through a black square and a horizontal line (95%CI). The aggregate effect size was displayed by the diamond at the bottom.

Publication bias

Publication bias was assessed using Egger's test. An intercept with $p<0.05$ indicates publication bias.

Duration of the study

The date of the first submission of this review protocol in PROSPERO was February 11, 2024, and the registration date in PROSPERO was February 22, 2024. Following that, starting from preliminary searches, data analysis, and manuscript writing took almost 3 months, i.e., March to May.

Results

Table 2 and Table 3 show various menstrual disorders among adolescent girls in rural and tribal areas. Both tables show sample size, age group, study design, and prevalence of various menstrual disorders found in rural and tribal study areas. A total of 61 studies (47 from rural, 14 from tribal areas, and 1 paper belonging to rural and tribal areas) among adolescent girls aged between 10 to 19 years in India have been evaluated and included in the analysis. In rural areas, sample size varied between 50 (Dharani and Sood, 2018) to 958 (Kohli and Kapoor 2021), whereas in tribal areas it varied between 100 (Nagar and Aimol 2010) to 507 (Shanmuganath et al. 2023).

Figure 3 shows the forest plot of the prevalence of dysmenorrhea. Each horizontal line with a square represents each included study. A total of 48 studies from rural and tribal adolescent girls in India were included in the stipulated time. The diamond at the bottom represents the overall results with a 95% confidence interval. The pooled prevalence of dysmenorrhea in rural areas was found the highest (89.33% with 95% CI: 80.05 to 95.27) by Das et al. (2019), while the lowest prevalence (12.80% with 95% CI: 10.74 to 15.08) was found by Kohli and Kapoor (2021). In tribal areas the highest prevalence of dysmenorrhea (78.30% with 95% CI: 74.45 to 81.81) was found in the study by Shanmugananth et al. (2023) and the lowest (15.80% with 95% CI: 11.71 to 20.63) was found by Kakeri et al. (2018). The overall prevalence of dysmenorrhea was 54.94% (95% CI: 47.93 to 61.85). Heterogeneity scores in this study were above 50% (Q-3374.99, DF-47, $p<0.0001$, I^2-98.61%), hence random effect model was considered. Figure 4 shows the funnel plot of effect size against sample size. Evidence of publication bias has been found through Egger's test ($p=0.0078$).

Figure 5 shows the forest plot of the prevalence of irregular menstruation. A total of 42 studies from rural and tribal adolescent girls in India were included. The diamond at the bottom represents the overall results with a 95% confidence interval. Analysis showed the highest prevalence of irregular menstruation in rural areas was found at 95.38% (95% CI: 92.49 to 97.39) by Ray et al. (2010), while the lowest prevalence was found at 5.60% (95% CI: 3.35 to 8.69) in the study by Kanotra et al. (2013). In tribal areas, the highest prevalence of irregular menstruation (32.30% with 95% CI: 28.24 to 36.56) was found in the study reported by Shanmugananth et al. (2023), and the lowest prevalence (12.60% with 95% CI: 7.74 to 18.99) was found by Kale et al. (2023). The random effect model showed that the prevalence of irregular menstruation was 26.21% (95% CI: 20.73 to 32.09). Substantial heterogeneity was found (Q-2232.65, DF-42, $p<0.0001$, I^2-98.12%). Figure 6 shows a funnel plot of the included studies and indicates no publication bias through Egger's test ($p=0.931$).

Table 2. Menstrual disorders among adolescent girls in rural areas

Study area	Studied sample size	Studied age group (in years)	Study design	Menstrual disorders								References
				Dysmen-orrhea	Irregular menstru-ation	PMS	Oligo-menor-rhea	Polymen-orrhea	Amenor-rhea	Menor-rhagia	hy-pomenor-rhea	
1. Garhwal	470	13–19	Cross-sectional	62.76%	28.72%	40.42%	-	-	-	9.50%	-	Negi et al. 2018
2. Amravati, Maharashtra	435	12–16	Prospective observational	62.30%	21.80%	17.90%	-	-	-	-	-	Wasnik et al. 2015
3. Tamil Nadu	500	14–19	Cross-sectional	65.00%	-	62.20%	16.00%	-	-	11.00%	-	Priya et al. 2016
4. Nagpur	146	12–16	Cross sectional	41.78%	-	34.90%	-	-	-	-	-	Thakre et al. 2012
5. Pondicherry	190	11–18	Cross-sectional	52.02%	24.00%	-	-	-	-	-	-	Karthiga et al. 2011
6. Lucknow	254	10–19	Cross-sectional	72.60%	18.10%	-	-	-	-	-	-	Sachan et al. 2012
7. Lucknow	176	1019	Cross-sectional	72.70%	18.10%	-	-	-	-	-	-	Sinha et al. 2016
8. Bhopal	400	12–19	Cross-sectional	33.75%	18.50%	-	8.00%	24.75%	-	-	-	Patel et al. 2023
9. Tamil Nadu	350	10–19	Cross-sectional	72.60%	31.70%	-	-	-	-	45.70%	-	Ravi et al. 2016
10. Bijapur	440	11–16	Cross-sectional	28.00%	7.50%	-	-	0.45%		5.90%	-	Patil and Angadi 2013
11. Wardha	171	10–19	Cross-sectional	45.61%	31.57%	65.50%	-	-	-	-	-	Dambhare et al. 2012
12. Varanasi	240	12–18	Cross-sectional	82.50%	37.00%	-	-	-	-	-	-	Nagar et al. 2022*
13. Dharwad, Karnataka	422	13–19	Cross-sectional	36.00%	-	-	-	-	-	-	-	Rajaretnam et al. 2010
14. Haryana	300	17–19	Cross-sectional	78.30%**	33.96%	-	16.67%	6.67%	2.33%	9.00%	-	Verma et al. 2021
15. Bengaluru	112	Below 20	Cross-sectional	48.00%	-	-	-	-	5.40%	4.50%	-	Anuradha and Manjunatha 2019
16. Maharashtra	100	10–19	Cross-sectional	78.00%	-	-	-	-	-	61.00%	-	Sharma et al. 2019

Study area	Studied sample size	Studied age group (in years)	Study design	Menstrual disorders								References
				Dysmenorrhea	Irregular menstruation	PMS	Oligomenorrhea	Polymenorrhea	Amenorrhea	Menorrhagia	hypomenorrhea	
17. Purba Midnapore, West Bengal	75	11–19	Cross-sectional	89.33%	-	-	-	-	-	12.00%	-	Das et al. 2019
18. Nagpur	600	10–19	Cross sectional	21.00%	-	-	13.00%	3.00%	-	7.00%	16.00%	Singh and Kasturwar 2017
19. North 24 Parganas, West Bengal	280	14–19	Cross-sectional	51.07%	92.14%	59.64%	-	-	-	-	-	Sanyal and Ray 2008
20. Hyderabad	120	11–16	Cross-sectional	-	28.00%	-	-	-	-	-	-	Kiran and Yashoda 2020
21. Haldwani	297	10–19	Cross-sectional	-	19.53%	-	-	-	-	-	-	Goyal 2018
22. Himachal Pradesh	111	-	Cross-sectional	-	19.80%	-	-	-	-	-	-	Walia et al. 2015
23. Ludhiana	958	10–19	Cross sectional	12.80%	-	-	6.70%	1.10%	1.90%	-	-	Kohli and Kapoor 2021
24. Maharashtra	122	13–19	Cross-sectional	65.60%	9.01%	-	-	-	-	-	-	Aggarwal et al. 2021
25. Patna, Bihar	300	13–17	Cross-sectional	74.70%	17.70%	-	-	-	-	-	-	Singh et al. 2023
26. Ludhiana, Punjab	50	13–18	Cross-sectional	62.00%		-	-	-	-	-	-	Dharani and Sood 2018
27. Maharashtra	620	10–19	Cross-sectional	44.20%	16.90%	-	-	-	-	-	-	Patil et al. 2009
28. Wardha	300	10–19	Cross-sectional	67.00%	-	-	-	-	-	-	-	Mudey et al. 2010
29. Kolkata, West Bengal	325	10–19	Cross-sectional	15.08%	95.38%	33.85%	-	-	-	-	-	Ray et al. 2010
30. Davanagar, Karnataka	200	10–19	Cross-sectional	56.50%	-	-	-	-	-	-	-	Basavaraju et al. 2019
31. Bangalore	190	12–17	Cross-sectional	52.60%	-	-	-	-	-	-	-	Kailashraj et al. 2020

32. Odissa	250	10–19	Cross-sectional	28.00%	-	-	27,00%	-	-	-	10.00%	Behera et al. 2017
33. Wardha	200	12–16	Cross-sectional	-	-	47.00%	-	-	-	-		Nimbhorkar et al. 2023
34. Marathewara, Maharashtra	122	13–19	Cross-sectional	54.10%	32.79%	-	-	-	-	-	-	Fatima et al. 2023
35. Prayagraj	500	15–19	Cross sectional	57.00%	16.00%	-	-	-	-	-	-	Saxena et al. 2023
36. Kerala	461	10–19	Cross-sectional	-	36.22%	-	-	-	-	-	-	Geroge and Sabita 2019
37. Raichur	80	13–16	Cross sectional	47.50%	16.30%	-	-	-	-	-	-	Ade and Patil 2013
38. Jaipur	180	10–19	Cross-sectional	41.66%	13.33%	-	-	-	-	25.00%	-	Yadav and Masand 2018
39. Nellore	169	11–16	Cross-sectional	71.60%	8.90%	-	-	-	-	-	-	Chinta et al. 2018
40. Karnataka	430	12–16	Cross-sectional	56.50%	33.02%	17.4%	-	-	-	-	-	Mann and Ts. 2023
41. Bhopal, Madhya Pradesh	350	10–17	Cross-sectional	-	42.30%	-	-	-	-	-	-	Mekle et al. 2020
42. Maharashtra	323	15–19	Cross-sectional	18.30%	5.60%	-	-	-	-	-	-	Kanotra et al. 2013
43. Kheda, Gujarat	200	13–18	Cross-sectional	62.00%	30.50%	-	-	-	-	-	-	Prajapati et al. 2015
44. Sabarkanta, Gujarat	250	13–18	Cross sectional	-	37.20%	-	-	-	-	-	-	Aggarwal et al. 2017
45. Mangaluru, Karnataka	132	13–18	Cross-sectional	-	30.32%	-	-	-	-	-	-	Senapathi and Kumar 2018
46. West Bengal	86	Below 20	Cross-sectional	48.3%	-	-	-	-	-	-	-	Lalbiaknungi et al. 2015
47. Kamrup, Assam	350	10–19	Cross-sectional	88.30%*	9.10%	-	-	-	-	-	-	Majhi and Das 2020

*Combined paper (1)

**Mild, moderate, and severe dysmenorrhoea was combined

Abbreviations: PMS: Premenstrual Syndromes

Table 3. Menstrual disorders among adolescent girls in tribal areas

Study area	Study sample	Studied age group (in years)	Study design	Menstrual disorders								Reference
				Dysmen-orrhoea	Irregular menstrua-tion	PMS	oligomen-orrhea	Polymen-orrhea	Amenor-rhea	Menor-rhagia	hypomen-orrhea	
1. Nagpur	290	10–19	Cross sectional	-	14.14%	-	-	-	-	-	-	Borkar et al. 2022
2. Balasore, Odissa	450	10–19	Cross sectional	28.88%	25.10%	79.11%	-	-	-	24.44%	90.00%	Mahapatra 2023
3. Achampet Mandal	425	10–19	Cross-sectional	25.00%	-	21.51%	-	18.98%	-	-	-	Sridhar and Gauthami 2017
4. Khunti, Jharkhand	150	13–18	Cross-sectional	-	30.66%	-	-	-	-	-	-	Kumari et al. 2021
5. Chittor, Andhra Pradesh	293	10–19	Cross-sectional	-	17.70%	-	-	-	-	-	-	Udayar et al. 2016
6. Mumbai	114	11–18	Cross-sectional	70.18%		-	-	-	-	-	-	Meshram et al. 2020
7. Amravati	150	Below 20	Cross-sectional, descriptive, and comparative	-	12.60%	-	-	-	-	-	-	Kale et al. 2023
8. Bhubaneswar, Odissa	300	10–16	Cross-sectional	52.00%	23.91%	-	-	-	-	-	-	Jena et al. 2017
9. West Garo hills of Meghalaya	100	13–18	Cross-sectional	97.00%	-	-	-	-	-	-	-	Nagar and Aimol 2010
10. Tamil Nadu	507	12–18	Cross-sectional	78.30%	32.30%	-	-	-	-	-	-	Shanmugananth et al. 2023
11. Jalpaiguri, West Bengal	301	10–19	Cross-sectional	78.07%	30.89%	89.70%	-	-	-	-	-	Thakur et al. 2020
12. Garo Hills of Meghalaya	240	12–18	Cross-sectional	62.5%	44.2%	-	-	-	-	-	-	Nagar et al. 2022*
13. Jammu and Kashmir	131	13–15	Cross-sectional	-	18.320%	-	-	-	-	-	-	Dhingra et al. 2009
14. Maharashtra	277	12–16	Cross-sectional	15.80%	23.40%	-	-	14.80%		11.50%	-	Kakeri et al. 2018

*Combined paper (2)

Abbreviations: PMS: Premenstrual Syndromes

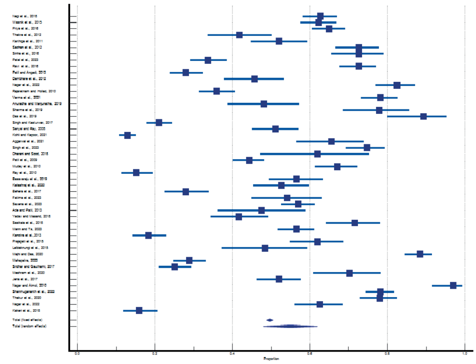

Figure 3. Forest plot of meta-analysis of proportion of dysmenorrhea

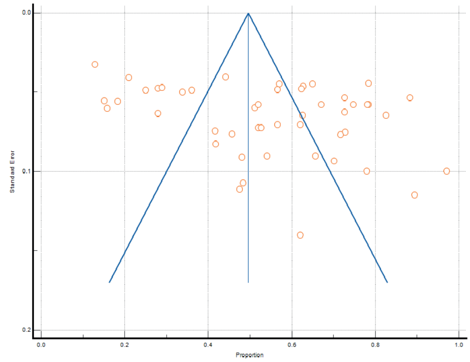

Figure 4. Funnel plot of meta-analysis proportion of dysmenorrhea

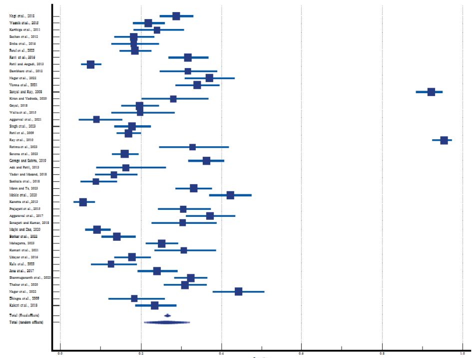

Figure 5. Forest plot of meta-analysis of the proportion of irregular menstruation

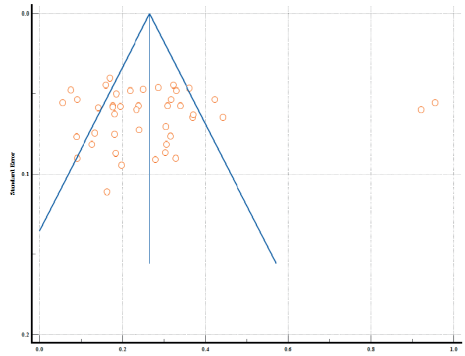

Figure 6. Funnel plot of meta-analysis proportion of irregular menstruation

Figure 7 shows the forest plot of 11 studies that reported PMS. Overall results were represented through the diamond at the bottom with a 95% confidence interval. The highest prevalence of PMS in rural areas was found to be at 65.50% (95% CI: 57.86 to 72.59) by Dambhare et al. (2012), and the lowest prevalence was found at 17.40% (95% CI: 13.93 to 21.32) by Mann and Ts. (2023). Only 3 studies were solely limited to tribal areas, among them the highest prevalence of PMS was found at 89.70% (95% CI: 85.69 to 92.89) by Thakur et al. (2020), while the lowest prevalence was found at 21.51% (95% CI: 17.69 to 25.72) by Sridhar and Gauthami (2017). The random effect model showed overall prevalence was 47.49% (95% CI: 31.44 to 63.81) and indicated a significant heterogeneity (Q-1105.30, DF-10, $p<0.0001$, I^2-99.10%). Figure 8 represents the funnel plot that indicates no publication bias was found by Egger's test ($p=0.637$).

Figure 9 depicts 6 studies reporting oligomenorrhea among rural areas. This disorder was not found in reported studies among tribal areas. The diamond at the bottom represents the overall results with a 95% confidence interval. The graph indicates that the highest prevalence of oligomenorrhea was found at 27.00% (95% CI: 21.59 to 32.95) by Behera et al. (2017) and the lowest prevalence was found at 6.70% (95% CI: 5.20 to 8.47) in a study by Kohli and Kapoor (2021). The random effect model of pooled prevalence was 13.88% (95% CI: 8.98 to 19.65). This estimate indicates a significant heterogeneity (Q- 89.34, DF-5, p<0.001, I^2- 94.40%). Egger's test showed no evidence of publication bias by funnel plot in Figure 10 (p= 0.056).

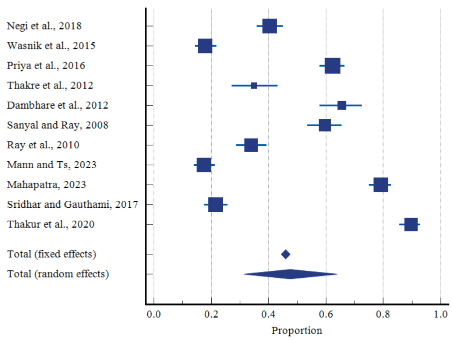

Figure 7. Forest plot of meta-analysis of proportion of PMS

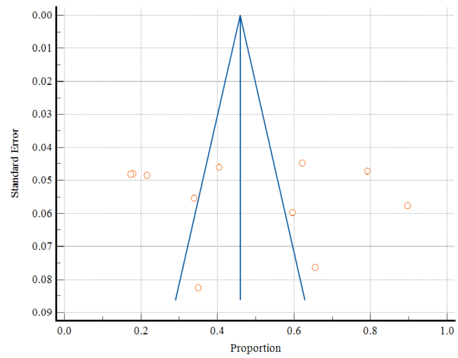

Figure 8. Funnel plot of meta-analysis proportion of PMS

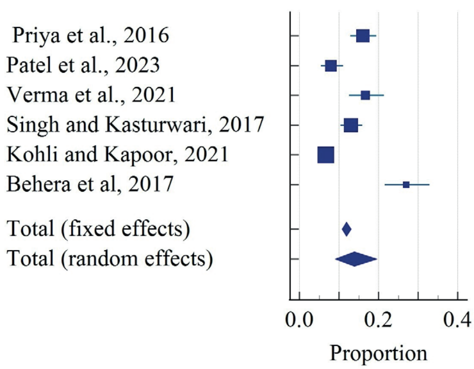

Figure 9. Forest plot of meta-analysis of the proportion of oligomenorrhea

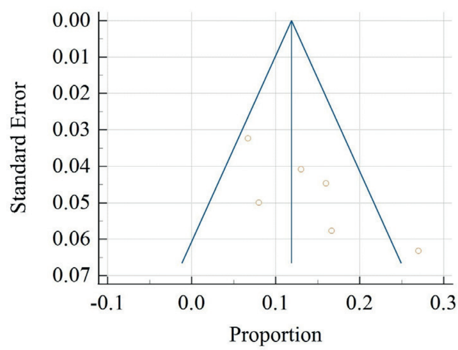

Figure 10. Funnel plot of meta-analysis proportion of oligomenorrhea

Figure 11 depicts a total of 7 studies that reported polymenorrhea, with only 2 belonging to tribal areas. Overall, results with a 95% confidence interval were represented through the diamond at the bottom. The highest prevalence in rural areas was found at 24.75% (95% CI: 20.59 to 29.28) by Patel et al. (2023), and the lowest prevalence was found at 0.45% (95% CI: 0.05 to 1.62) in a study by Patil and Angadi (2013). In tribal areas the highest prevalence of polymenorrhea was found at 18.98% (95% CI:15.36 to 23.03) by Sridhar and Gauthami (2017), and the lowest prevalence was found at 14.80% (96% CI: 10.83 to 19.53) by Kakeri et al. (2018). The overall prevalence of polymenorrhea was 7.85% (95% CI: 2.30 to 16.31) estimated by the random effect model which showed a significant heterogeneity (Q-356.45, DF-6, P<0.0001, I^2-98.32%). There was no evidence of publication bias by Egger's test (p=0.130; Fig. 12).

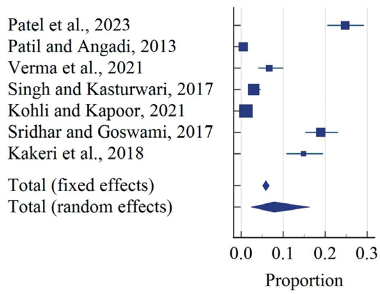

Figure 11. Forest plot of meta-analysis of proportion of polymenorrhea

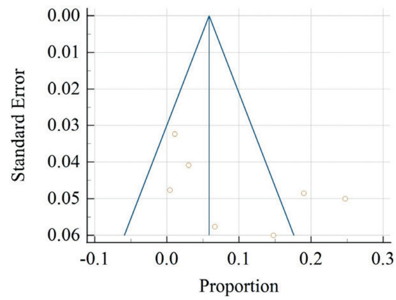

Figure 12. Funnel plot of meta-analysis proportion of polymenorrhea

Figure 13 shows a total of 12 studies indicating menorrhagia with only 2 belonging to tribal areas. The diamond at the bottom represents the overall results with a 95% confidence interval. The highest prevalence in rural areas was found at 61.00% (95% CI: 50.73 to70.59) in a study by Sharma et al. (2019), and the lowest prevalence was found at 4.50% (95% CI: 1.55 to 9.90) by Anuradha and Manjunatha (2019). In tribal areas the highest prevalence of menorrhagia was found at 24.40% (95% CI:20.53 to 28.68) among adolescent girls of various tribal communities by Mahapatra (2023), while the lowest prevalence was found at 11.50% (95% CI: 7.99 to 15.85) by Kakeri et al. (2018). The random effect model showed the overall prevalence of menorrhagia at 16.83% (95% CI: 10.04 to 24.96). A significant heterogeneity was present (Q-431.03, DF-11, P<0.0001, I^2-97.45%). There was no evidence of publication bias by Egger's test (p=0.321; Fig. 14).

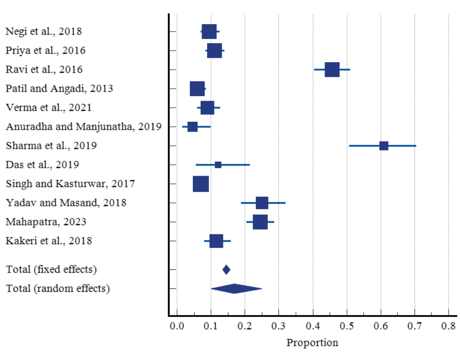

Figure 13. Forest plot of meta-analysis of proportion of menorrhagia

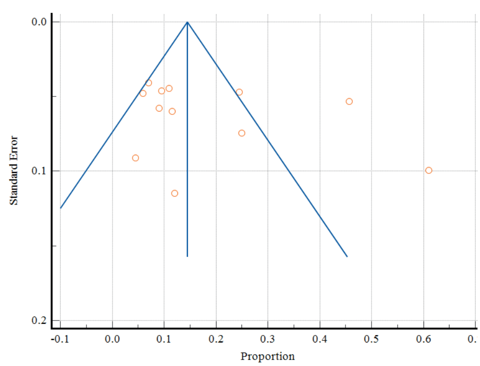

Figure 14. Funnel plot of meta-analysis proportion of menorrhagia

Menstrual disorders and associated factors

Table 4 shows that, among 61 reviewed articles, only 17 (12 from rural and 5 from tribal areas) reported associated factors with menstrual disorders among adolescent girls. Table 4 shows major associated factors of menstrual disorders were lack of physical activities, dietary habits, BMI, socio socioeconomic factors among rural and tribal adolescent girls in India.

Table 4. Associated factors of menstrual disorders among adolescent girls in rural and tribal areas

Study area	Study sample	Studied age group (in years)	Study design	Associated factors	Reference
Rural areas					
1. Garhwal	470	13–19	Cross-sectional	Junk food Lack of physical activity	Negi et al. 2018
2. Tamil Nadu	500	14–19	Cross-sectional	BMI	Priya et al. 2016
3. Lucknow	254	10–19	Cross-sectional	Age	Sachan et al. 2012
4. Haryana	300	17–19	Cross-sectional	Nutritional status Dietary habits Socio-economic factors	Verma et al. 2021
5. Maharashtra	100	10–19	Cross-sectional	Age factors of the participants	Sharma et al. 2019
6. Nagpur	600	10–19	Cross-sectional	Age of the adolescents Education of mothers Education of the respondents BMI	Singh and Kasturwar 2017
7. North 24 Parganas, West Bengal	280	14–19	Cross-sectional	Age groups Socio-economic factors Occupation and education of parents	Sanyal and Ray 2008
8. Ludhiana	958	10–19	Cross-sectional	Age factors	Kohli and Kapoor 2021
9. Kolkata, West Bengal	325	10–19	Cross-sectional	Socio economic variables	Ray et al. 2010
10. Kerala	461	10–19	Cross-sectional	Age factors	Geroge and Sabita 2019
11. Karnataka	430	12–16	Cross-sectional	Clinico-Socio-demographic factors	Mann and Ts 2023
12. West Bengal	86	Below 20	Cross-sectional	Socio-economic group BMI	Lalbiaknungi et al. 2015
Tribal areas					
13. Bhubaneswar, Odessa	300	10–16	Cross-sectional	Socio-economic factors	Jena et al. 2017
14. West Garo hills of Meghalaya	100	13–18	Cross-sectional	Age of the respondents Total family income	Nagar and Aimol 2010

Tribal areas					
Study area	Study sample	Studied age group (in years)	Study design	Associated factors	Reference
15. Tamil Nadu	507	12–18	Cross-sectional	Lack of knowledge and awareness Cultural taboos and restrictions Poor nutrition and socio-economic factors	Shanmugananth et al. 2023
16. Jalpaiguri, West Bengal	301	10–19	Cross-sectional	BMI Educational status of participants Skipping the menstrual cycle	Thakur et al. 2020
17. Jammu and Kashmir	131	13–15	Cross-sectional	Low level of knowledge regarding menstruation Mother's attitude towards menstruation	Dhingra et al. 2009

Abbreviations: BMI: Body Mass Index

Discussion

The present review systematically summarizes the prevalence of menstrual disorders among adolescent girls living in rural and tribal areas in India. In rural areas, the highest prevalence of dysmenorrhea (89.33%) was found in Purba Midnapore, West Bengal by Das et al. (2019). This finding are in line with a questionnaire-based study conducted among 757 Malaysian adolescent girls, showing 85.7% of adolescents experienced dysmenorrhea out of which 42.1% have moderate dysmenorrhea and 11.2% have severe dysmenorrhea (Azhary et al. 2022). 88.0% of adolescent girls were experiencing dysmenorrhea in Portugal (Marques et al. 2022). 80.4% of Swedish adolescents were experiencing dysmenorrhea (Gambadauro et al. 2024). Irregular menstruation (95.38%) was found highest in Kolkata, West Bengal by Ray et al. (2010). A study conducted among 106 rural Nepali found that 76.6% of adolescent girls were having irregular periods (Chhetri and Singh 2020). Whereas the lowest prevalence of oligomenorrhea (6.70%) and amenorrhea (1.90%) was found in Ludhiana by Kohli and Kapoor (2021). These results were quite similar to the survey reported by Aryani et al. (2018) conducted among 444 Indonesian adolescent girls, which showed that 24.5% of the girls experienced oligomenorrhea, polymenorrhea was found among 5.9% of girls and only 0.2% of the girls were experiencing amenorrhea. On the contrary, the highest prevalence of oligomenorrhea (27.00%) and the lowest prevalence of hypomenorrhea (10.00%) were found in Odisha (Behera et al. 2017). Another significant finding includes Amenorrhea which was found the highest (5.40%) and menorrhagia was found the lowest (4.50%) in Bengaluru (Anuradha and Manjunatha 2019). PMS (Pre-menstrual syndromes) was higher (65.50%) in Wardha, Maharashtra (Dambhare et al. 2012), and the lowest prevalence (17.40%) was reported in Karnataka by Mann and Ts (2023). In Sweden, 1100 adolescent girls had at least one menstrual problem reported as either moderate (81.3%) or severe (31.3%) followed by mood swings (81.1%) (Gambadauro et al. 2024). The lowest prevalence of dysmenorrhea (15.80%), polymenorrhea (14.80%), and menorrhagia (11.50%) were reported in Maharashtra (Kakeri et al. 2018). On the contrary, the highest prevalence of menorrhagia (24.44%) was reported among adolescent girls from various tribal communities (Mahapatra 2023). The highest prevalence of dysmenorrhea (78.30%) and irregular menstruation (44.2%) was found in the West Garo hills of Meghalaya (Nagar and Aimol 2010; Nagar et al. 2022). The lowest prevalence of PMS (21.50%) and the highest prevalence of polymenorrhea (18.90%) were reported in Achampet Mandal, Andhra Pradesh (Sridhar and Gauthami 2017). In addition, the highest prevalence of PMS (89.70%) was found among the adolescents of Jalpaiguri district of West Bengal (Thakur et al. 2020). A study conducted by Sharma et al. (2016) in the Pokhara Valley of Nepal, showed that 64.2% of the girls had irregular menstruation followed by oligomenorrhea (23.1%). Another study conducted in Northwest Ethiopia reported that 75.4% of the adolescents were experiencing PMS (Zegeye et al. 2009). The prevalence of oligomenorrhea and amenorrhea was not found in these studies.

This review reveals an overall prevalence of dysmenorrhea, irregular menstruation, PMS, oligomenorrhea, polymenorrhea, and menorrhagia among adolescent girls in rural and tribal areas and the meta-analysis shows the prevalence was 54.96% (95% CI: 47.93 to 61.85), 26.21% (95% CI: 20.73 to 32.09), 47.49% (95% CI: 31.44 to 63.81), 13.88% (95% CI: 8.98 to 19.65),7.85% (95% CI: 2.30 to 16.31), 16.83% (95% CI: 10.04 to 24.96) respectively. Similar results were reported in the review done by Samani et al. (2018) among Iranian adolescent girls, showing the overall pooled prevalence of dysmenorrhea, oligomenorrhea, polymenorrhea, and menorrhagia was 73.27% (95% CI: 65.12 to 81.42), 13.11% (95% CI: 10.04 to 16.19), 9.94% (95% CI: 7.33 to 12.56%) and 19.24% (95% CI: 12.78 to 25.69). Prevalence of PMS and irregular menstruation was not reported in the review. Based on a large Italian data, 6.7% (95% CI: 5.4% to 7.0%) and 9.0% (95% CI: 7.7% to 9.4%), 3.0% (95% CI: 2.5% to 3.4%) of adolescent girls were suffering from dysmenorrhea, irregular menstruation, and polymenorrhea, oligomenorrhea, and menorrhagia was found among 3.4% (95% CI: 2.9% to 3.9%) and 19.0% (95%CI: 17.9% to 20.1%) of adolescent girls respectively (Rigon et al. 2012).

This review also tried to highlight as much as possible about the factors associated with menstrual disorders. Dysmenorrhea and irregular menstruation were associated with food habits while PMS was associated with a lack of physical activities (Negi et al. 2018) which can lead to school absenteeism (Priya et al. 2016). Considerable pain during menstruation affects daily activities among adolescent girls in Southwestern Nigeria (Amu and Bamidele 2014). School absenteeism was also due to the menstrual pain among adolescent girls in Nepal (Sharma et al. 2016). BMI was found to have a strong association with these disorders (Jena et al. 2017; Priya et al. 2016; Singh and Kasturwar, 2017; Thakur et al. 2020; Verma et al. 2021) and similar results were reported by Bahadori et al. (2023) among Iranian adolescent girls. The age of the respondents plays an important role in menstrual-related problems (Sanyal and Ray 2008; Nagar and Aimol 2010; Singh and Kasturwar 2017; George and Sabita

2019; Sharma et al. 2019; Kohli and Kapoor 2021). Late adolescents were more likely to have menstrual irregularities (Singh and Kasturwar 2017). The age of the respondents was found to have a significant association with menstrual disorders among adolescent girls in other countries as well (Marques et al. 2022; Paudel 2022). For example, studies found that socioeconomic factor is another significant factor associated with various menstrual disorders (Sanyal and Ray 2008; Ray et al. 2010; Nagar and Aimol 2010; Lalbiaknungi et al. 2015; Jena et al. 2017; Verma et al. 2021; Shanmugananth et al. 2023). Socioeconomic status was also associated with menstrual disorders in Nepal and Sweden (Chhetri and Singh, 2020; Gambadauro et al. 2024). Lack of knowledge about menstruation was associated with menstrual irregularities among Gujjar tribal adolescent girls (Dhingra et al. 2009). Another study among tribal adolescent girls in Tamil Nadu found that low socio-economic status, lack of knowledge, and poor nutrition can be responsible for the high prevalence of primary dysmenorrhea, and Cultural restrictions and taboos play a major role during menstruation. Adolescents often hesitate to talk about their menstruation-related problems due to cultural restrictions and taboos and, as a result, the ability to bear menstrual pain in girls increases (Shanmugananth et al. 2023). Mothers often consider menstruation as a negligible issue, for that reason most mothers are found to be reticent to their daughters regarding menstruation and its related issues (Dhingra et al. 2009). Similarly, mother's education was found to have a strong connection among adolescent girls in Nepal and has major restrictions on religious or family activities (Chhetri and Singh 2020). The previous study found that PMS was associated with some clinico-socio-demographic factors, such as age, class, menstrual cycle regularity and duration, and menstrual flow which results in increasing depression and anxiety among them (Mann and Ts 2023). Similar results were reported among Iranian adolescent girls (Bahadori et al. 2023).

Limitations of the study

Meta-analysis with small studies (<5) can lead to narrow confidence intervals and estimation of heterogeneity is quite difficult in this situation which can result in a biased effect (Mathes and Kuss 2018). Hence, two disorders (amenorrhea and hypomenorrhea) were exempted from meta-analysis due to unavailability of more than 5 articles. Only 3 articles were found to have these two disorders. This study is limited to English-language publications. Authors have tried as much as possible to read all the relevant articles that indicate menstrual disorders among adolescent girls in rural and tribal areas in India. Some relevant articles might have been missed because it is humanly impossible to read all the articles.

Conclusion

This study shows the overall prevalence of menstrual disorders i.e., dysmenorrhea, irregular menstruation, PMS, oligomenorrhea, polymenorrhea, and menorrhagia among adolescent girls in rural and tribal areas in India. This review reveals that the most predominant disorders were dysmenorrhea, irregular menstruation along menorrhagia in both areas. Adolescents refused treatment due to shame and discomfort. Lack of physical activities, dietary habits, BMI, socio socioeconomic factors were linked to menstrual irregularities among adolescent girls in India. The study also revealed that adolescent girls in both areas at least have one problem related to menstruation and the prevalence of these menstrual disorders varies in different areas all over India. Adolescent girls in rural and tribal areas in India were in a vulnerable situation during menstruation.

Future scope

This review is one of the first attempts to study menstrual disorders among adolescent girls specifically in rural and tribal areas in India. With this review, we have tried to provide information related to the overall prevalence of menstrual disorders and associated factors among them in both areas. However, future research needs to be done on this specific aspect. Awareness should be taken at home as well as in schools. Proper guidance on every aspect of menstruation should be arranged before and after attained menarche. Due to low socio-economic status, many girls may not have access to healthcare or any treatment, so health camps should be organized in different rural as well as tribal areas.

Abbreviations

BMC – BioMed Central. CASP – Critical Appraisal Skills Programme, PRISMA-Preferred Reporting Items for Systematic Reviews and Meta-Analysis, PROSPERO – International Prospective Register of Systematic Reviews, UNICEF – United Nations Children's Emergency Fund, WHO – World Health Organization.

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Conflict of interest

none

Author's contribution

SC gave the idea. RC conceptualized and designed the whole study. Registration of the review, literature reviews, quality assessment of studies, data analysis was done by RC and then discussed with SC. RC wrote the whole manuscript and checked it by SC. All authors have read, checked, and approved the whole manuscript.

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A comparative analysis of height trend and nutrition among 1983 to 2005 birth cohort of Ilorin metropolis, Kwara State, Nigeria

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Abstract: Over the course of the years, numerous studies have been conducted to assess the trends in height across different generations and regions. This study seeks to add to the current data on secular height trend, by analyzing the trend in height, gender specific differences in height trend and relationship between socio-nutritional factors and increase in height trend among 1983–2005 birth cohort in the Ilorin metropolis. A total of 414 study participants aged 18–40 years (207 males and 207 females). Their height was obtained using the Tape stadiometer, information about dietary history (nutritional factors) that may affect height were gotten via the use of Questionnaires. Data were analyzed using the Statistical Package for Social Sciences (SPSS) Version 27.0 and results were computed using Pearson's Chi-square analysis and ANOVA. P-values less than 0.05 were considered significant. Height was found to have positively increased among the 1983 to 2005 birth cohorts of Ilorin metropolis with the most significant increase being observed in the Males. This study also revealed a positive correlation between Diary and Carbohydrate consumption and increase in height, especially among males. No significant increase in height was found among females of the birth cohorts and no Association was found between the considered nutritional factors and female height.

Key words: height trend, nutrition, birth cohort, Ilorin

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Introduction

In the field of Anthropology, there is a significant emphasis on tracing evolutionary trends in the human body. Numerous studies have been conducted to investigate whether specific characteristics of the human body have undergone evolutionary changes over time. Over the course of civilization, numerous studies have been conducted to assess the trends in height or stature across different generations and regions.

In anthropometry, height refers to the measurement of a person's stature, which is the distance from the bottom of the feet to the highest point of the head. This assessment is conducted directly using a stadiometer. Measuring height is important for studying physical growth patterns and secular trends across regions and population groups, as population-specific height assessments can help identify areas of concern and target populations.

Ilorin, the capital city of Kwara State, Nigeria is located in the North – Central region of Nigeria. It is a significant urban center serving as a meeting point for various ethnic groups, such as Yoruba, Fulani, and Hausa (National Population Commission 2018). The residents of Ilorin metropolis primarily engage in a mix of agricultural activities, trading and small-scale industries. The diet in Ilorin is largely based on locally available foods, reflecting the agricultural and animal rearing practices of the region. Staple food includes various forms of carbohydrates, such as rice, yam, maize, complemented by proteins from poultry, cattle and Dairy products (Adebayo 2020). Dairy products, particularly in the form of milk and locally made cheese (Wara), is common, especially among the cattle herders (Oloyede 2019). Ilorin's environment is characterized by a tropical climate with distinct wet and dry seasons, which supports the cultivation of a variety of crops. The fertile land and favorable weather conditions enable the production of food items that form the basis of the local diet. This diet is generally rich in carbohydrates, providing the energy necessary for the physically demanding activities that many residents engage in. In addition, the consumption of dairy products, which are rich in calcium, plays an important role in the nutritional status of the population, contributing to the overall health and growth of the residents, particularly during childhood and adolescence (Adebayo 2020).

Globally, human height has increased steadily over the past 200 years with the average person today being taller compared to their ancestors living one hundred years ago. This trend is consistent with improvements in general health and nutrition during this time. The main aim of this research is to determine whether there is a significant difference in height trend between generations in Ilorin, as well as to analyze gender specific differences in height trend within the studied generation and to identify the relationship between nutrition history and any observed shifts in stature.

Several scholars have studied secular trends in height across various countries and regions in the world using, typically, two main data sources: conscript measurements and self-reported data from certain databases (Quintana-Domeque et al. 2012). Spijker et al. (2008) made analysis based on self-reported data, which revealed that height significantly increased throughout the 20th century. For example, individuals born in the 1980s were about 10 cm taller than those born in the 1910s.

Several studies using conscript data, including works by Martínez-Carrión (2005), indicate a consistent increase in the average adult stature of male conscripts in Spain from 160 cm in 1900 to 175 cm in the early 1990s. In a research on the evolution of height carried out by Quintana-Domeque et al. (2012) across Spanish regions, it was reported that the average heights of females and males born 1950 and 1980 increased by 1.7 cm and 1.6 cm per decade, respectively. Garcia and Quintana-Domeque (2007) carried out similar research on the evolution of adult height in 10 European countries for cohorts born between 1950 and 1980. Height data from Austria, Belgium, Denmark, Finland, Greece, Ireland, Italy, Portugal, Spain, and Sweden were collected and analyzed and it was reported that height increased in all the listed countries although countries from Southern Europe (Greece, Italy, Portugal, and Spain) experienced greater increase in height and stature compared to the Northern Europe (Austria, Belgium, Denmark, Finland, Ireland, and Sweden).

According to Komlos and Baur (2004), factors such as improved nutrition, healthcare, and living standards contribute to these positive height trends in many regions. Baird (2015) also stated that the small gain in average human height experienced in many countries over the last few hundred years was not due to genetic evolution. The most likely cause is improved nutrition and health as nutrition, specifically the quality of the diet, plays a huge role and is one of the strongest determinants of human height (Perkins et al. 2016).

Victoria et al. (2008) in their study emphasized the fact that adequate nutrition during crucial growth periods is critical for optimal height attainment, and malnutrition can lead to stunting. Grasgruber et al. (2014) found that the ratio of high-quality animal proteins, such as those from milk products, red meat, and fish, is the best indicator of male height in high-income countries where there is a high intake of animal protein. A study by Zhao et al. (2020) reported that males consumed more dietary energy, fats, proteins, and carbohydrates than females. Similarly, another study reported that males exhibited a pronounced preference for red meat and processed meats, while females showed a higher propensity to consume vegetables, whole grains, tofu, and dark chocolate (Feraco et al. 2024). A study of geographic differences in stature among young men from 45 countries of European origin demonstrated that nutrition level explained most of the differences in adult height, particularly the consumption of high-quality proteins from milk, pork, fish, and wheat (Grasgruber et al. 2014).

The study conducted by Takahashi (1984) found a significant association between nutrition and height by demonstrating a link between Japan's height trend to increased milk consumption since the Second World War. Similarly, milk consumption was positively associated with increase in adult height among a nationally representative sample from the United States (Wiley 2005). In addition, it was shown that the production and consumption of milk and beef provides insights into why Germanic people living outside the Roman Empire were taller compared to those residing at its core (Moradi and Baten 2005). This may be due to the fact that Milk contains calcium, which is an essential mineral for bone growth and elongation which contributes to an adult overall height (stature).

Height, as a comprehensive anthropometric indicator, offers a holistic view of a population's overall well-being, that consideres both historical and contemporary factors that shape human development. The primary aim of this study is to investigate generational shifts in stature among Ilorin residents born between 1983 and 2005, with a focus on understanding how height has changed over time.

Focusing specifically on the birth cohort between 1983 and 2005, this study aims to contextualize the influences shaping height trends among the residents of Ilorin, Kwara State, Nigeria, considering factors such as shifts in dietary patterns and lifestyle. This research also endeavors to enrich the existing body of knowledge by providing a comprehensive examination of generational shifts in stature among Ilorin Residents born between 1983 and 2005 and aspires to offer a nuanced understanding of the complex factors shaping human growth patterns over time. This research could also help predict generational shift in human stature in the future, factors that may contribute to it and contribute to the overall understanding of the secular trends in human height across generations over time.

Material and Methods

Two hundred and Fourteen subjects from the Ilorin Metropolis, Kwara State, Nigeria (comprising one hundred and seven males and one hundred and seven females) were used for this research. All measurements were taken by the same examiner (to reduce inter-observer error). Participants were healthy and within the age range of 18–40 years. Participants with severe physical health conditions that might affect stature (osteoporosis, spinal deformities, and amputated legs), cognitive impairments and who were born before 1983 or after 2005 were excluded from this study.

Ethical approval was obtained from the University Ethical Review Committee (UERC) for human experimentation of the University of Ilorin, Ilorin, Kwara State, Nigeria and this study was approved with ethical approval number UERC/ASN/2024/2758 on the 14th of March, 2024. All participants consented to participate in this research experiment.

Questionnaires were administered to collect information about demographic details (nationality, age, and sex), dietary history and stature.

The questionnaires contain structured questions about the participants typical diet during their formative years (childhood and adolescence) and questions about the frequency of consumption of specific food groups (dairy, protein and carbohydrates).

For the measurement of height, the participants were asked to stand barefooted with their back straight and upright against a flat surface after adequate explanation of the procedure is done since none of the procedure is invasive, neither harmful nor painful to the participants. The height measurement was taken using a stadiometer and measured in centimeters (cm).

The Statistical Package for Social Sciences (SPSS) Version 27.0 was used. Results were computed using Pearson's chi-square analysis and ANOVA method. P-values less than 0.05 were considered significant.

Results

Table 1 presents the trend in height among different birth year cohorts. It appears that the cohorts born between 1997–1999 and 2000–2002 have the highest average height with mean values of 169.20 cm and 169.82 cm respectively along with relatively low SD (8.16 cm and 8.24 cm). Following closely are the cohorts born between 1983–1986 and 2003–2005, with mean heights of 167.84 cm and 167.66 cm, respectively, and slightly higher standard deviations (6.14 cm and 8.65 cm). The cohort born within 1994–1996 has a slightly lower mean height of 166.97 cm but a higher standard deviation of 9.88 cm. The cohorts born between 1987–1989 and 1990–1993 have the lowest mean heights of 165.88 cm and 165.45 cm, respectively, with standard deviations of 7.15 cm and 6.98 cm. In addition, the provided p-value of 0.044 suggests that there is a statistically significant difference in mean height between at least two of the birth year cohorts (as it is <0.050). This indicates that there are indeed differences in height among the various birth year cohorts.

Table 1. Observed height difference between the considered birth cohorts in Ilorin

Birth Years	Height Difference (N=414)			ANOVA	
	Mean (SD)	Min	Max		
	(cm)	(cm)	(cm)	F-value	Sig.
1983–1986	167.84 (6.14)	156.00	179.00	2.180	**0.044**
1987–1989	165.88 (7.15)	156.00	179.00		
1990–1993	165.45 (6.98)	152.00	178.00		
1994–1996	166.97 (9.88)	153.50	200.70		
1997–1999	169.20 (8.16)	152.60	188.60		
2000–2002	169.82 (8.24)	154.00	187.50		
2003–2006	167.66 (8.65)	137.10	189.00		

Note: *SD= Standard deviation, N= Number of cases, Min= Minimum, Max= Maximum, F-value= Fisher's value*

Table 2. Height difference between the considered birth cohorts in Ilorin (Gender Based)

Birth Year	Height Difference			ANOVA	
	MALE (N=207)				
	Mean (SD)	Min	Max	F-value	Sig.
	(cm)	(cm)	(cm)		
1983–1986	169.95 (5.22)	157.00	179.00	5.390	**<0.001**
1987–1989	168.61 (7.90)	156.00	179.00		
1990–1993	168.83 (5.72)	159.00	178.00		
1994–1996	174.70 (7.98)	168.00	200.70		
1997–1999	174.70 (5.96)	166.00	188.60		
2000–2002	175.07 (6.03)	165.00	187.00		
2003–2006	173.87 (6.92)	157.50	189.00		

Table 2. (cont.)

Birth Year	Height Difference			**ANOVA**	
	FEMALE (N=207)				
	Mean (SD) (cm)	Min (cm)	Max (cm)	F-value	Sig.
1983–1986	164.87 (6.24)	156.00	176.90	1.330	0.245
1987–1989	163.33 (5.46)	156.00	171.00		
1990–1993	161.57 (6.32)	152.00	176.00		
1994–1996	160.11 (5.20)	153.50	170.00		
1997–1999	163.70 (6.11)	152.60	175.00		
2000–2002	163.69 (5.94)	154.00	175.00		
2003–2006	162.66 (6.36)	137.10	177.00		

Note: *SD= Standard deviation, N= Number of cases, Min= Minimum, Max= Maximum, F-value= Fisher's value*

Table 2 presents height differences among males born in different birth year cohorts. It shows that the cohorts born within 1983–1986 have a mean height difference of 169.95 cm with a standard deviation of 9.95 cm. The cohorts born between 1987–1989 and 1990–1993 have slightly lower mean height of 168.61 cm and 168.83 cm, with standard deviations of 7.90 cm and 5.72 cm, respectively, indicating less variation in height differences within these groups. On the other hand, the cohorts born between 1994–1996 and 1997–1999 have higher mean height differences, both with a mean of 174.70 cm and standard deviations of 7.98 cm and 5.96 cm, respectively. The cohort born within 2000–2002 has the highest mean height difference of 175.07 cm, with a standard deviation of 6.03 cm, while the cohort born within 2003–2005 has a mean height difference of 173.87 cm with a standard deviation of 6.92 cm. The p-value of 0.001 suggests that there are statistically significant differences in height differences among the various birth year cohorts. In the table above, it can be observed that there was a gradual in the height trend of the males down the birth cohorts, this indicates that the differences in height observed between the cohorts are unlikely to be due to random chance and are likely meaningful.

Table 2 also presents height differences among females born in different birth year cohorts. It reveals that the cohorts born within 1983–1986 have the highest mean height difference, with a value of 164.87 cm and a standard deviation of 6.24 cm. Conversely, the cohorts born between 1990–1993 and 1994–1996 exhibit the lowest mean height differences, with values of 161.57 cm and 160.11 cm, respectively, and relatively low standard deviations (6.32 cm and 5.20 cm), indicating less variation in height differences within these groups. The cohorts born between 1987–1989, 1997–1999, and 2000–2002 have mean height differences slightly higher than the lowest groups, with mean values ranging from 163.33 cm to 163.69 cm and standard deviations ranging from 5.46 cm to 6.11 cm. The cohort born within 2003–2005 has a mean height difference of 162.66 cm with a standard deviation of 6.36 cm. The p-value of 0.245 suggests that there are no statistically significant differences in height differences among the various birth year cohorts (as it is >0.050). Instead, there seems to be some sort of fluctuations and reduction in the height trend among the birth cohort of the females.

Table 3. Pearson's chi-square analysis between Stature and considered nutritional history in the General population

	HEIGHT	VARIABLES	X^2
	df	p-value	
Diary Consumption	134.007 [a]	125	0.275
Protein Consumption	131.576[a]	125	0.326
Carbohydrates Consumption	158.913[a]	125	**0.022**

Note: *df= degree of freedom,* X^2 *=Chi square*

Table 3 shows that carbohydrate consumption has a statistically significant relationship with stature, indicated by a p-value of 0.022. This suggests that there is a significant association between carbohydrate consumption and stature in the study sample. On the other hand, the other nutritional factors, such as dairy and protein consumption did not show a statistically significant relationship with stature, as their p-values were greater than 0.050. This implies that these factors did not have a significant impact on stature in the research findings.

Table 4. Chi square analysis between stature and nutrition history in the general population (Gender based)

VARIABLES	MALES (stature)		FEMALES (stature)	
	X^2	p-value	X^2	p-value
Diary Consumption	98.705	**0.041**	21.392	1.000
Protein Consumption	79.949	0.356	76.602	0.459
Carbohydrates Consumption	136.640	**<0.001**	29.740	1.000

Note: X^2 *=Chi square*

Based on the information included in Table 4, the chi-square analysis of the association between stature and socio-economic factors, such as dairy, protein, and carbohydrate consumption in males and females revealed significant associations with carbohydrate and dairy consumption in males only. Specifically, the p-values for carbohydrate and dairy consumption in males were 0.001 and 0.041, respectively, indicating a significant association. In contrast, the p-values for all factors in females were greater than 0.050, suggesting no significant association. Overall, the findings of the chi-square analysis suggest that carbohydrate and dairy consumption may have a more significant impact on stature in males compared to females.

Discussion

In this study, height was found to have positively increased among the 1983 to 2005 birth cohorts of Ilorin metropolis. This agrees with the findings of Garcia and Quintana Domeque (2007) showing that there is indeed increase in height trends across several generations according to their study on the evolution of adult height between 1950 to1980 birth cohort in 10 European countries. This study also supports the study by Garenne (2020) which noted increase in height trend of Sub-Saharan and North African countries. According to a global study by NCD Risk factor collaboration (2016), it was noted that Mean height for males increased from 162 cm to 171 cm, and for females, it increased from 151 cm to 159 cm. But in this study, it was observed that the mean height for males within the 1983 to 2005 cohorts increased from 169.95 to 173.87, while that of the females showed no significant difference but fluctuates between 164 cm to 162 cm. The same increase in mean height of males was also observed in a study by Cole (2003) and Kolodziej et al. (2015). Also, according to Martinez Carrion (2005), there is a consistent increase in the average adult height of males from 160 cm to 175 cm based on his study on male conscripts in Spain, which according to this study was found to be a mean height increase of 169 cm to 172 cm. This study agrees with the reports of Jelenkovic et al. (2016) based on their research on the 1886 to 1994 cohorts, which noted that there is generally an increase in height trend with the mean height being greater in the males than females.

According to Roser et al. (2024), population wide distribution of height is influenced by non-genetic environmental factors such as Nutrition, Health and Lifestyle.

In this study, an association was observed between the increase in height and nutritional factors such as carbohydrate and dairy consumption. This finding is consistent with the reports by Baird (2015), who suggested that improved nutrition is a likely driver of height increase. In addition, the study supports the findings of Komolos and Baur (2004), indicating that factors such as nutrition and lifestyle contribute to a positive trend in height.

This study contradicts the findings of Allen and Uauy (1994), who claimed that the consumption of protein such as meat, eggs and dairy products positively correlates with height. However, this study observed that only the consumption of dairy products showed a significantly positive correlation with height increase, particularly in males. Interestingly, protein consumption was found to have no significant effect on height increase in both males and females.

This study also presents findings that correlates with the reports of Moradi and Baten (2005), Takahashi (1984), and Wiley (2005), which advocate for the notion that the increase in height is primarily attributed to increased milk or dairy consumption. Similar to these assertions, the results obtained from this research indicates a positive correlation between dairy consumption and height increase.

According to Moradi and Baten (2005), diets dependent on carbohydrates consumption such as rice, negatively correlates with increase in height trend. However, this study contradicts that finding, revealing that carbohydrate consumption is positively correlated with height in males, while showing no significant correlation with height in females.

These findings suggest that carbohydrate and dairy consumption may have a more significant impact on stature in males compared to females. This could be due to various factors, such as differences in dietary patterns, nutrient absorption, and metabolism between genders.

It is also worth noting that previous studies have reported gender differences in dietary patterns and nutrient intake. For instance, a study found that males consumed more dietary energy, fats, proteins, and carbohydrates than females ($p < 0.001$) (Zhao et al. 2020). Similarly, another study reported that males exhibited a pronounced preference for red meat and processed meats, while females showed a higher propensity to consume vegetables, whole grains, tofu, and dark chocolate (Feraco et al. 2024). These differences in nutrient intake could contribute to the observed gender differences in the relationship between stature and nutritional factors.

The findings of this study underscore the importance of understanding gender-specific nutritional needs and how they contribute to physical development. For researchers, this suggests that future studies should explore the underlying biological mechanisms and potential gender differences in nutritional impacts. In practice, the results could inform public health strategies aimed at improving dietary guidelines for adolescents, particularly in regions similar to Ilorin, to optimize growth and development outcomes.

Conclusion

This study demonstrated evidence that there is increase in height trend in males and that certain nutritional factors correlates with increase in height, with the males having a stronger correlation with carbohydrates and diary consumption than the females among Ilorin residents born 1983–2005. The observed increase in male height according to the findings of this research project may be due to its association with dairy and carbohydrate consumption as dairy products, rich in calcium, are essential for bone growth and elongation which contributes to height, and a diet rich in carbohydrates provides the necessary energy for growth processes and nutrient absorption. Together, these factors promote bone growth and development and support the proper functioning of growth plates, contributing to the increase in height observed. The lack of an increase in the height trend of the female birth cohort may also be attributed to the negative association that exists between increase in height trend and the nutritional history considered in this research.

There is need for further research involving a larger population in order to firmly establish these findings. With regards to the findings on the positive association between increase in height trend and socio-nutritional factors, more studies should be carried out to explore the underlying mechanisms and potential implications of these findings.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Authors' contribution

Alabi Ade Stephen – principal investigator and initiator of the research work; Olagunju Peace Eyitayo – Data collection; Alabi

Adeola, Owa Joshua Abidemi, Owa Yetunde Elizabeth – Data analysis and interpretation; Ayoola Abosede Mary, Dare Ezekiel Babatunde – Manuscript preparation; Olasehinde Oluwatoyin Ezekiel, Adeoye Titilayo Temitope – Manuscript editing.

All authors contributed the revision and proofreading of the manuscript.

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Elliptical Fourier analysis of molar outlines in Late Pliocene *Parapapio whitei* from Makapansgat Limeworks, South Africa

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Abstract: Introduction: Alongside *Australopithecus africanus* at Makapansgat South Africa, dated to nearly 3 million years before present, are remnants of *Parapapio* (Cercopithecinae). The extreme variability of this fossil assemblage has stymied efforts to specify the taxon parameters for *Parapapio,* which are attributed to at least three species.

Study aims: The first maxillary molar occlusal outlines of the two most complete fossils attributed to *Parapapio whitei* are compared. The degree of group cohesion in *Parapapio whitei* is evaluated using three extant cercopithecoid taxa.

Methods and Materials: The fossil crania from Makapansgat Members 3–4, MP 221 and MP 223, both referred to *Parapapio whitei*, are compared to three extant cercopithecoid taxa including *Cercocebus agilis* (n=8), *Colobus angolensis* (n=8) and *Papio anubis* (n=8). Molar shape is captured using elliptical Fourier analysis of occlusal outlines and molar size dimensions are estimated from measuring software.

Results: MP 223 is larger than MP 221 in occlusal area and the minimum buccolingual length of M^1 although the variability between the two *Parapapio whitei* fossils is commensurate with that observed in *Papio anubis*. MP 221 and MP 223 are more similar to one another in occlusal outline shape than to any other taxon. However, MP 223 falls consistently closer to *Papio anubis* whereas MP 221 resembles *Papio anubis* in some respects and *Cercocebus agilis* in others.

Conclusion: MP 221 and MP 223 likely belong to a single species with no clear affinity to any of the extant taxa examined. The differences in molar size characterizing *Parapapio whitei*, a terrestrial forager, is potentially indicative of male bimaturatism or ecological variability which may also characterize *Australopithecus africanus* at Makapansgat.

Key words: MP 221, MP 223, *Cercocebus agilis*, *Colobus angolensis*, *Papio anubis*, *Australopithecus africanus*

Original article

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Introduction

There are at least four cercopithecoid monkeys of the genus *Parapapio* fossilized in the karstic caves systems of South Africa that yield the remains of early hominins attributed to *Australopithecus africanus*, ranging from approximately 2.9 to 2.0 Ma before present (BP). These monkey fossils include *Parapapio whitei*, found primarily at Makapansgat and Sterkfontein, alongside *Parapapio jonesi* and *Parapapio broomi* (Fig. 1). A fourth species from South Africa, *Parapapio antiquus*, has been historically reported at Taung, but may be *Procercocebus* (Gilbert 2007), or another taxon resembling *Cercocebus* spp. (Szalay and Delson 1979) (Fig. 1). *Parapapio* largely becomes extinct in southern Africa around 2 Ma BP with the exception of *Parapapio jonesi*, which survived the transition to a cool dry environment, and is found in the deposits yielding *Paranthropus robustus* (Brain 1981; Elton 2007; Williams et al. 2007, cf. Frost et al. 2022).

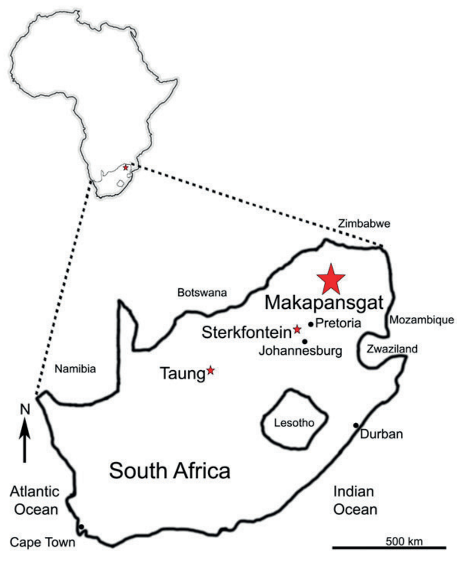

Figure 1. Location of Makapansgat Cave in South Africa, shown enlarged from a map of Africa

Several approaches have emerged to categorize the fossils attributed to *Parapapio*. Since the fossilized remains of *Parapapio* are incomplete, consisting primarily of skull or gnathic fragments encased in breccia, the craniofacial complex and dentition have figured heavily in these investigations (Freedman 1957; 1960; 1976; Maier 1970; Delson 1992; Heaton 2006; Williams et al. 2007; Gilbert 2013). Earlier assessments of *Parapapio* species attribution relied on the size of the molars, placing Sterkfontein *Parapapio whitei* as the largest, *Pp. jonesi* the smallest while *Pp. broomi* was associated with intermediate values (Freedman and Stenhouse 1972). However, the majority of authors have been equivocal about species attribution within *Parapapio* (cf. Gear 1926; Jones 1937; Broom and Jensen 1946; Freedman 1957; 1960; 1976; Maier 1970; Freedman and Stenhouse 1972; Eisenhart 1974; Szalay and Delson 1979; Delson 1992; Jablonski 2002; Heaton 2006; Williams et al. 2007; Fourie et al. 2008). Nevertheless, *Parapapio whitei* appears to present the largest cranial dimensions (Williams et al. 2007) and larger body sizes (Delson et al. 2000) compared to the other southern African species attributed to *Parapapio*. Given its larger size, *Parapapio whitei* may have been partly terrestrial, exploiting plants close to ground level or possibly underground storage organs reflected in the mixed C3/C4 isotope signal of this species, at least at Makapansgat (Fourie et al. 2008). Dental microwear texture of *Parapapio whitei* fossils at Makapansgat, such as MP 223, suggests hard and brittle items as well as adhering grit from underground storage organs were consumed, potentially accounting for the C4 contribution to the diet (Williams 2014). In contrast, the smaller species *Pp. jonesi* may have had a more folivorous diet at Makapansgat (El-Zaatari et al. 2005).

The nine *Parapapio whitei* fossils from Makapangat, out of a total of 144 cercopithecoids recovered from the site (Freedman 1976), have been featured in isotopic, dietary, scaling and morphometric studies. Whether any two *Parapapio whitei* fossils are within the limits of an extant cercopithecoid taxon needs to be addressed prior to examining the attribution of fossils to species. However, as the fossils are fragmentary, comparisons involving morphological size and shape can be challenging.

We chose to examine the molar crown as an indicator of size and shape given the phylogenetic importance of teeth in fossil and living primates. Molar morphology, as well as maximum dimensions of the occlusal surface, are shown to be highly heritable considering the fact that cusp morphology is heavily influenced by genetic factors (Hlusko et al. 2002; 2006; Monson and Hlusko 2014; Paul and Stojanowski 2017). The shape of the occlusal margin of molars is influenced by the maximum and minimum dimensions of the cusps that differ in size per individual and can be effectively captured using elliptical Fourier analysis.

Elliptical Fourier analysis

A variety of methods have been developed to describe the shape of the molar crown. These include the use of a generalized Procrustes analysis followed by geometric morphometrics (Gómez-Robles et al. 2007). Generalized Procrustes superimposition compares sets of landmarks to calculate a mean shape rather than a comparison of all landmarks to an arbitrarily designated shape as in elliptical Fourier analysis (Corny and Détroit 2014). Linear distances have also been employed to describe the shape of molars (e.g., Pilloud and Larsen 2011). The scoring of traits using the Arizona State University Dental Anthropology System (Turner et al. 1991; Scott and Irish 2017) is an additional methodological tool invented to describe and compare dental morphology across individuals. These approaches require relatively unworn teeth. Approximating crown form by tracing or landmarking the margin is yet another method to capture the morphology of the molars. In contrast to other approaches, elliptical Fourier analysis of molar occlusal outlines can include individuals with a slight degree of attrition since minor dental wear does not affect the molar margin (Brophy et al. 2014).

Elliptical Fourier analysis creates a numeric description of the shape of an object fitted to a curve by comparing individuals to an idealized sphere (Fig. 2). The deviations between the empirical and standard closed objects are estimated using sine and cosine to calculate the spatial differences between the overlapping ellipses. An outline conforms to the standardized ellipse by increasingly accurate shape modifications. The process continues until the molar outlines start to resemble the standardized ellipse. The sum of these perturbations is quantified into the amplitudes of the harmonics. Each harmonic explains a unique aspect of the shape of an object. Therefore, the more complex the shape, the greater the number of harmonics is needed to describe the object. Any shape can be characterized using elliptical Fourier analysis if the object has a closed contour and is rendered in two dimensions (Lestrel 1974; 1989; Kuhl and Giardina 1982; Iwata and Ukai 2002). A closed contour that is circular or oval, like molars, can be fitted with relatively fewer amplitudes of the harmonics (Ferrario et al. 1999; Corny and Détroit 2014; Williams et al. 2017; 2021).

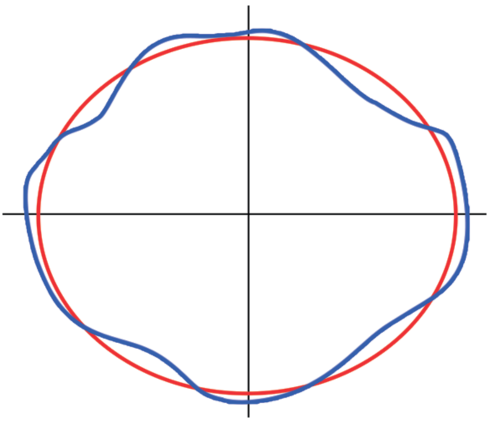

Figure 2. Idealized ellipse (red) compared to a sample (blue) demonstrates how elliptical Fourier functions estimate the difference between standard and empirical closed contours

Purpose of the study

Another advantage of elliptical Fourier analysis over Procrustes and other morphometric approaches is the ability to compare shapes regardless of size. In a study by Corny and Détroit (2014), Procrustes superimposition and elliptical Fourier analyses were employed to differentiate first and second human molars. Both tools are effective at differentiating individuals into groups (Corny and Détroit 2014; see also Claude 2013). The absence of size or centroid size in the output from elliptical Fourier analysis is critical in this study as the size of the molars of terrestrial monkeys, such as *Papio anubis*, and some *Parapapio* fossils are two to three times larger than those of smaller cercopithecine monkeys, such as *Cercocebus agilis* or African colobine monkeys, such as *Colobus angolensis*. In this study we utilize elliptical Fourier analysis on the first maxillary molar outlines of MP 221 and MP 223, the two most well preserved, least worn *Parapapio whitei* fossils from Makapansgat. We ask whether the range of variation between the two fossils attributed to *Parapapio whitei* are commensurate with that observed in extant cercopithecoid monkeys, including close and more distantly related genera. We expect MP 221 and MP 223 to be distinct from colobine monkeys such as *Colobus angolensis*, and probably more similar to *Papio anubis* than to *Cercocebus agilis*. The inclusion of a species attributed to the Colobinae serves as an outgroup and reflects the fossil cercopithecoid fauna at the site given the presence of the colobine monkey species, *Cercopithecoides williamsi*, at Makapansgat.

Materials and Methods

Materials

Occlusal outlines and measurements of the first permanent maxillary molar from *Parapapio whitei* fossils MP 221 and MP 223 were compared to three Old World monkey taxa. These included olive baboons, *Papio anubis* (n=8) and agile mangabeys, *Cercocebus agilis* (n=8), which are both cercopithecine monkeys and closely related to *Parapapio* (Szalay and Delson 1979). We also included Angolan black and white colobus monkeys, *Colobus angolensis* (n=8) from the Colobinae, which is more distantly related to *Parapapio*. All three comparative taxa derive from the Democratic Republic of the Congo and were molded at the Royal Museum for Central Africa in Tervuren, Belgium.

The fossil *Parapapio whitei* remains were examined at the University of the Witwatersrand in Johannesburg, South Africa. Only individuals free of substantial dental attrition, postmortem defects or casting artifacts were included in the study. There are potentially nine relatively well preserved *Parapapio whitei* fossil crania with gnathic elements from Makapansgat. These include MP 47, MP 76, MP 117, MP 119, MP 221, MP 223, MP 239, M3133 and M3147 (Tab. 1). However, some are reconstructed or damaged areas obscure the taxonomic attribution, while others exhibit extensive dental attrition (Tab. 1).

Table 1. Makapansgat fossil primate crania potentially attributable to *Parapapio whitei*

Museum#	Museum attribution	Observations
MP 47	not listed	Probable *Pp. whitei* male given the tall snout, elevated nasal bones and broad palate; teeth heavily worn
MP 76	*Pp. whitei*	Maier (1970) and Gilbert et al. (2018) refer to *Pp. broomi*; Freedman (1960, 1976) attributes the cranium to *Pp. whitei*
MP 117	*Pp. whitei*	Inferior muzzle with large heavily worn molars
MP 119	*Pp. broomi/ whitei*	Probable *Pp. whitei* female based on the tall snout and raised nasal bones; extensive dental attrition
MP 221	*Pp. whitei*	Large well preserved male cranium attributed to *Pp. whitei*; upper phase 1 breccia west quarry
MP 223	*Pp. whitei*	Large well preserved male cranium attributed to *Pp. whitei*; upper phase 1 breccia west quarry
MP 239	*Pp. whitei*	Likely to be *Pp. whitei* given the raised nasal bridge; the small molars suggest a female individual, although M^1 and M^2 exhibit heavy attrition and preservation damage
M3147	not listed	Probable *Pp. whitei* given the raised nasal bones and tall snout but also shows affinities to fossil *Papio* sp. from the inflated maxillary fossae
M3133	not listed	Small male, probable *Pp. whitei*; large and deep palate like other *Pp. whitei*; molars are damaged and heavily worn

Context of the *Parapapio whitei* fossil remains

The individuals ascribed to *Parapapio whitei*, MP 221 and MP 223, are considered males (Freedman 1976; Williams et al. 2007, Gilbert 2013) and both derive from the upper phase 1 breccia west quarry and were recovered in 1973, corresponding to Members 3–4, but are likely associated with the most fossiliferous zone of the cave consisting of grey breccia and referred to as Member 3. It is also possible that Members 3 and 4 were deposited at overlapping temporal intervals albeit Member 3 may have been more wooded or a shift in the accumulating predators occurred, which likely included hyenas, large felids, porcupines and birds of prey (Reed 1997; Latham et al. 2007; Arbor 2010). Most of the fossils from Makapansgat have no known locality data since they derive from breccia piles left behind during commercial mining operations. In the middle of the 19th century, Makapansgat valley in Limpopo Province of northeast

South Africa (Fig. 1) was the site of a Boer raid on a Ndebele tribe whose chief named *Mokopane* was ambushed near the cave, or *gat* in Afrikaans (Reed et al. 2022). Bovid fossils from Makapansgat Limeworks were sent to Raymond Dart in 1925 after his famous Taung child was publicized, and some 20 years later, Phillip Tobias and his students collected cercopithecoid fossils from these same breccia piles (Arbor 2010; Reed et al. 2022). When the cercopithecoid fossils from Makapansgat were compared to those recovered from Sterkfontein Member 4 the antiquity and contemporaneity of the two caves was evident to Dart, Tobias and others (Reed et al. 2022). Although dating the Sterkfontein deposits remains mired in uncertainty (cf. Herries et al. 2013; Frost et al. 2022; Granger et al. 2023), Makapangat is probably older given the absence of *Papio* (Maier 1970; Freedman 1976; Delson 1984; 1988; Jablonski 2002) and other faunal seriations between sites (Vrba 1999; 2000; Reed 1997; Reed et al. 2022).

When the site was formed, Makapansgat was wooded with open areas and presented a mosaic ecology with diverse albeit archaic fauna such as *Parapapio whitei* (Vrba 1996; 2000; Reed 1997; Elton 2007; Reed et al. 2022). Biochronological estimates using bovid, suid and cercopithecoid fauna date Members 3–4 at Makapansgat to approximately 2.85–2.50 Ma (Herries et al. 2013). A biochronology using cercopithecids gives an estimated date of 2.7–2.6 Ma (Frost et al. 2022) which is identical to a magnetostratigraphy and biochronology study by Warr (2009). Another analysis of paleomagnetism suggests a range of 3.03 to 2.58 Ma (Herries et al. 2013).

Morphology of *Parapapio*

Parapapio can be distinguished from *Papio* by the lack of an ante-orbital drop characterizing the rostrum of extant baboons. The muzzle in *Parapapio* as viewed in *norma lateralis* forms a steep slope from glabella to prosthion when preserved (Szalay and Delson 1979; Jablonski 2002). An additional contrast to *Papio* is the limited degree of sexual dimorphism in *Parapapio* as well as the smaller overall cranial size (Freedman 1957). The anterior temporal lines are suggested to converge in *Parapapio* at least in some individuals (Gilbert 2007). *Parapapio* lacks sagittal cresting and development of the supraorbital torus is minimal; maxillary and mandibular fossae are diminutive or small; and the hypoconulid of M_3 is reduced among other distinguishing traits, such as a tall muzzle and raised nasal bones (Gear 1926; Jones 1937; Broom and Jensen 1946; Freedman 1957; 1960; 1976; Maier 1970; Eisenhart 1974; Szalay and Delson 1979; Delson 1992; Jablonski 2002; Heaton 2006; Williams et al. 2007; Gilbert et al. 2018). The teeth are also distinctive in *Parapapio* as the incisors and molars are smaller than those of *Theropithecus darti* and larger than those of *Cercopithecoides williamsi*, at least at Makapansgat (Maier 1970: 89, Table III).

Morphological descriptions of MP 221 and MP 223

MP 221 comprises a relatively complete cranium from a young adult male, first described by Freedman (1976). Superiorly on the muzzle, the nasal bones are raised. The size of the cranium is relatively large as are the relatively unworn molars, particularly M^2 and M^3. The canines must have also been impressively large judging from the expansive empty crypts.

MP 223 is probably older than MP 221 given the slightly heavier dental attrition. MP 223 consists of a nearly complete cranium that lacks the superior roof as noted by Freedman (1976). There is a steep facial angle and raised nasal bones typifying *Parapapio*. The molars are comparatively large. Like MP 221, the rostrum is relatively tall. The canines are also large and somewhat baboon-like.

Methods

Dental molds of M^1 were created using polyvinylsiloxane, President Jet regular body (Coltène-Whaledent). At the Georgia State University Bioarchaeology Laboratory, the dental molds were placed into putty cradles, catalyzed with activator, prior to being filled with the centrifuged epoxy resin and hardener solution. The dental casts were allowed to cure for at least 24 hours before being pried from the molds.

Data capture

Toupview® was used to digitize images of the dental casts which were magnified at 30x. Molar occlusal area was approximated using the polygon-tracing tool in which a series of points were placed along the border of molar-to-molar contact to complete an outline (Fig. 3). To standard the images, the first landmark to be digitized was the most lingual aspect of the lingual groove and, continuing clockwise, additional landmarks were placed totaling between 50–60 points. To assess intraobserver error, individuals from each species were traced several times. The resulting differences between tracing attempts were minimal.

The area of the digitized molar outline created in Toupview was calculated using the measurement function. In addition, mesiodistal and minimum buccolingual measurements of the crown were also obtained using the digitized outline. For mesiodistal length of the first maxillary molar, a linear distance between the most mesial and distal extremes was calculated. For the minimum buccolingual distance, the measurement tool was used to draw a line between the most lingual extreme of the intersection of the protocone and hypocone to the midpoint of the buccal margin. A measurement error study was conducted in which two attempts at collecting the measurements were compared using a t-test which yielded nonsignificant differences between trials.

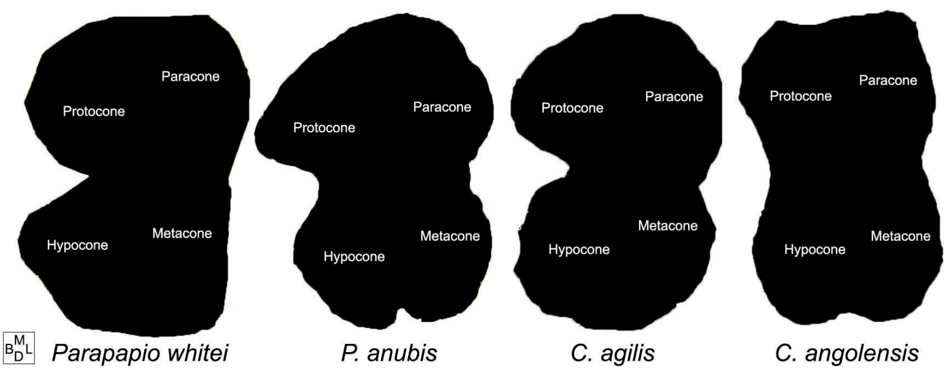

Figure 3. Binarized occlusal outline images of *Parapapio whitei* (MP 221) compared to *Papio anubis*, *Cercocebus agilis* and *Colobus angolensis* showing the approximate location of the major maxillary cusps on cercopithecoid molars

The digitized outlines from Toupview were imported into photo editing software to binarize the images and to vertically flip any right molars that were traced in place of more poorly preserved left ones. The binarization contrasts the area within the outline, which was rendered black, with the outside of the outline, which was filled with white (Fig. 3).

Analytical methods

All of the binarized images (n=26) were subjected to elliptical Fourier analysis within the SHAPE v.2.0 program (Iwata and Ukai 2002). Principal component scores were extracted from the amplitudes of the harmonics to represent molar occlusal outline shape in further analyses. The mean values plus and minus two standard deviations for each principal components vector were visualized to demonstrate the variability of the sample. For the measurements, descriptive statistics for the extant taxa and the values for MP 221 and MP 223 were compared and size differences were further explored in a bivariate plot of mesiodistal and minimum buccolingual lengths. The first PC score, explaining the greatest amount of variation, was contrasted with occlusal area as well as with the second PC axis. In order to encompass a summary of occlusal molar shape variation, a discriminant function analysis was performed on the PC scores identified as significant from the elliptical Fourier descriptors. With a sample size of eight for the comparative taxa, only the first seven PC scores were included so as not to violate the assumptions of the discriminant function analysis. An added benefit of utilizing discriminant function analysis was to classify *Parapapio whitei* vis-à-vis the extant taxa and to calculate Mahalanobis distances. The first two canonical scores and Mahalanobis distances for MP 221 and MP 223 to all taxa were compared. In all these analyses, the comparative taxa are shown with convex hulls encompassing 100% of the variation in the sample.

Results

Out of 76 PC scores generated in SHAPE, 10 PC scores are identified as significant via elliptical Fourier analysis. There is a sharp decrease in the amount of variance explained after PC5 (5.1%) as demonstrated by the variation around the mean shape of the occlusal outlines (Fig. 4). The amount of variance explained ranges from 34.3% for PC1 to 1.65% for PC10. The first 10 PC scores account for 94%, whereas the first seven explain 87.8% of the total occlusal molar shape variance.

Differences in molar size dimensions

The means and standard deviations for *Cercocebus agilis*, *Colobus angolensis* and *Papio anubis* are compared to the fossil values for mesiodistal and minimum buccolingual lengths and occlusal areas (Tab. 2). Individuals ascribed to *Parapapio whitei* are larger than *Cercocebus agilis* and *Colobus angolensis* for all measurements and are smaller than the *Papio anubis* mean for mesiodistal length. However, MP 223 rivals the minimum buccolingual mean and exceeds the mean occlusal area for *Papio anubis.* In contrast, MP 221 is smaller than *Papio anubis* across the board (Tab. 2).

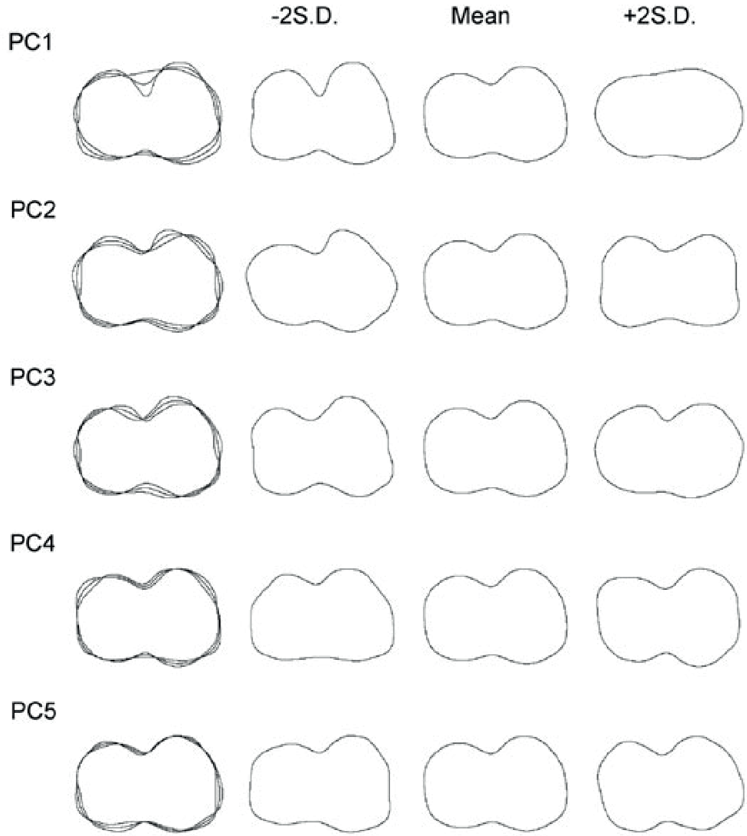

Figure 4. Mean occlusal outlines, plus and minus two standard deviations (S.D.), for the first five PC axes inclusive of 81% of the variance in the sample

Table 2. *Parapapio whitei* measurements, with the means (standard deviations) for the comparative taxa

Taxon	Mesiodistal	Minimum Buccolingual	Occlusal area
Parapapio whitei (MP 221)	9.98	4.46	58.91
Parapapio whitei (MP 223)	10.01	5.77	81.71
Cercocebus agilis	7.018 (0.363)	3.084 (0.321)	26.238 (4.208)
Colobus angolensis	6.186 (0.275)	3.247 (0.166)	22.571 (1.968)
Papio anubis	11.632 (0.755)	5.715 (0.484)	78.444 (11.577)

The comparison between minimum buccolingual and mesiodistal lengths show that M^1 dimensions attributed to *Parapapio whitei* are much larger than observed in *Cercocebus agilis* and *Colobus angolensis* (Fig. 5). *Papio anubis* exhibits larger dimensions than the other taxa, although MP 223 comes close to the values attributed to *Papio anubis*. MP 221 and MP 223 are quite similar in mesiodistal length but differ substantially in minimum buccolingual dimensions. However, a similar degree of variation between individuals is observed in *Papio anubis* (Fig. 5).

PC axis 1 compared to occlusal area

When PC1 is contrasted to occlusal area, all of the taxa fall within discrete ranges, including *Parapapio whitei* represented by MP 221 and MP 223 (Fig. 5). With respect to shape of the maxillary first molar occlusal surface represented by PC1 (34.3%), the difference between the two fossil papionins is relatively small and both fall closest to one *Cercocebus agilis* individual. This is particularly true of MP 223, which is also similar to a single *Papio anubis*. Although the difference between the two *Parapapio whitei* fossils is comparatively large for occlusal area, the same disparity of values can be observed in *Papio anubis*. Occlusal area can be considered a contrast vector polarizing *Colobus angolensis* and *Papio anubis*. Across this axis, MP 221 is only similar to a single *Papio anubis* while MP 223 is similar to several (Fig. 5).

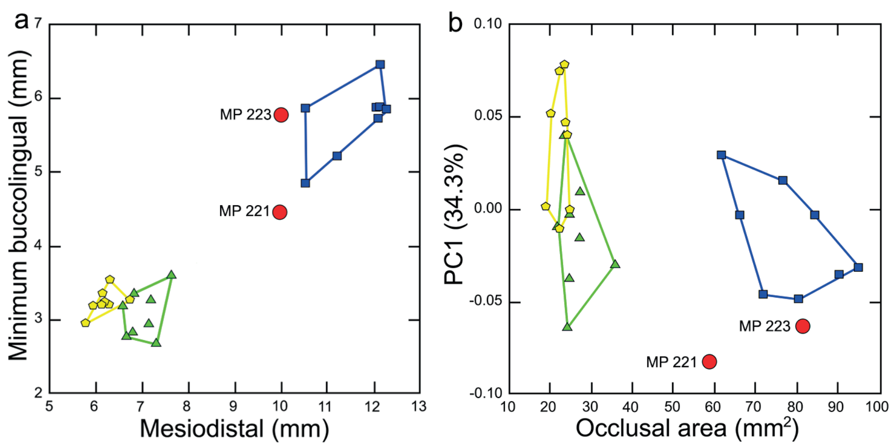

Figure 5. Comparison of (a) minimum buccolingual and mesiodistal M[1] lengths and (b) PC1 and occlusal area for *Parapapio whitei* (red circles); *Papio anubis* (blue squares); *Cercocebus agilis* (green triangles) and *Colobus angolensis* (yellow hexagons)

PC axes from elliptical Fourier analysis

The first two principal components scores, explaining a total of 52.7% of the variance, completely separate *Parapapio whitei* from the convex hulls encompassing 100% of the variation in each comparative taxon (Fig. 6). On the first axis, accounting for about a third of the shape variance (34.3%) MP 221, and to a lesser extent MP 223, are separated from *Colobus angolensis*. Most of the two extant cercopithecines hover close to zero suggesting they are difficult to classify. However, at least one *Papio anubis* individual falls close to MP 223 suggesting some similarities in shape. In addition, two *Cercocebus agilis* individuals are close to MP 223 on PC1. However, these same *Cercocebus agilis* monkeys are polarized from MP 221 on PC2, explaining 18.4% of the variance. Also on the second PC axis, one *Colobus angolensis* individual approximates the projection of MP 221 whereas MP 223 is undifferentiated from the comparative taxa (Fig. 6).

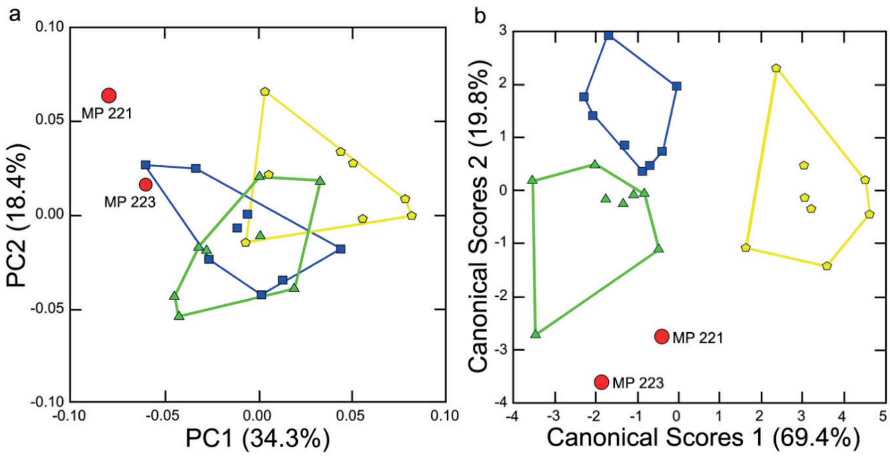

Figure 6. Comparison of (a) the first two PC scores explaining 52.8% of the variance and (b) canonical scores axes 1 and 2 using 7 PC scores for *Parapapio whitei* (red circles); *Papio anubis* (blue squares); *Cercocebus agilis* (green triangles) and *Colobus angolensis* (yellow hexagons)

Discriminant function analysis of 7 PC scores

The two *Parapapio whitei* fossils are relatively close to one another and no further away from each other than the extremes of the comparative taxa (Fig. 6). On Canonical Scores 1, with an eigenvalue of 5.7 and explaining 69.4% of the variance, MP 221 and MP 223 are both distinct from *Colobus angolensis*. However, MP 221 overlaps *Cercocebus agilis* and *Papio anubis*. In contrast, MP 223 is similar only to *Cercocebus agilis*. On Canonical Scores 2 with an eigenvalue of 1.6 and accounting for 19.4% of the shape variation, both MP 221 and MP 223 are decidedly unlike *Papio anubis* and also differ from *Colobus angolensis.* In fact, MP 223 is unlike any of the extant taxa on Canonical Scores 2, while MP 221 slightly overlaps one *Cercocebus agilis* (Fig. 6).

Squared Mahalanobis distances for MP 221 and MP 223 compared to all other taxa demonstrate how well each of these fossil papionins corresponds to their taxon and the relative weights attached to the differences with other taxa. MP 221 and MP 223 exhibit the lowest squared Mahalanobis distances of 5.4 for *Parapapio whitei* (Table 3). This is much lower than the double digit D^2 distances describing the difference between MP 221 and MP 223 and all the comparative taxa. Predictably, the highest D^2 distances are between the *Parapapio whitei* fossils and *Colobus angolensis* whereas the lowest squared Mahalanobis distance is between *Cercocebus agilis* and MP 221 of 16.5 (Tab. 3). Relatively low D^2 distances also exist between MP 223 and *Papio anubis* (25.2) and *Cercocebus agilis* (26). The posterior probabilities for group membership of the Makapansgat fossils with respect to the three comparative taxa are all <0.001. The squared Mahalanobis distances agree with the classification rates in which 96% of individuals are correctly classified. This includes MP 221 and MP 223 which are both classified as *Parapapio whitei*. However, total jack-knifed classification rates are lower (65%) and one *Parapapio whitei* is classified as *Cercocebus agilis* (MP 221) and another as *Papio anubis* (MP 223).

Table 3. Squared Mahalanobis distances for MP 221 and MP 223 compared to all taxa

	C. agilis	*C. angolensis*	*P. anubis*	*Pp. whitei*
MP 221	16.5	42.9	28.1	5.4
MP 223	26.0	33.2	25.2	5.4

Discussion

We asked whether the morphology captured via occlusal outlines and measurements of the first maxillary molar of MP 221 and MP 223 could be encompassed within a single species of cercopithecoid monkeys. We also asked whether MP 221 and MP 223 are more similar to *Papio anubis* or to *Cercocebus agilis*, the two taxa more closely related to early papionins, compared to more distantly related colobine monkeys represented by *Colobus angolensis*.

MP 221 and MP 223 are similar mesiodistally but differ substantially in minimum buccolingual dimensions and occlusal area. The difference in molar size between MP 221 and MP 223 has been commented on previously by Freedman (1976) who first described these two fossils. In fact, the difference was so pronounced with respect to other cercopithecoid comparisons that Freedman (1976) equivocated as to whether MP 221 was actually *Parapapio whitei*, proposing the possibility that this fossil might also be *Pp. broomi*. However, a similar molar size difference between individuals is seen in *Papio anubis* (Fig. 5) suggesting MP 221 and MP 223 could be members of the same species. The same could be said for shape (Fig. 6). None of the resulting size and shape comparisons of MP 221 and MP 223 with the three cercopithecoid species refute the association of these two males as belonging to a single taxon.

The taxonomy of the genus *Parapapio* has come under scrutiny. For example, *Parapapio antiquus* could be a subspecies of *Pp. broomi* (Heaton 2006) or a different genus, *Procercocebus*, purportedly ancestral to the *Cercocebus* clade (Gilbert 2007). Shape resemblances between *Parapapio* and *Papio* (Freedman 1957) and *Parapapio* and *Cercocebus* spp. (Szalay and Delson 1979) have been noted previously. With respect to the results obtained here, the question of whether MP 221 and MP 223 are likely to be ancestral to *Papio* typified by *Papio anubis* or a *Cer-*

cocebus clade represented by *Cercocebus agilis* is uncertain. MP 223 is clearly more aligned with *Papio anubis* in some ways, but does not fall within the convex hull for this species in size and shape indices (Fig. 5 and 6) and squared Mahalanobis distances present significant differences with extant taxa. The case for MP 221 is more complex in that there is some similarity in occlusal outline shape of M^1 with *Cercocebus agilis* and perhaps, to a lesser degree, with *Papio anubis*. *Parapapio whitei* and other taxonomic labels given to the fossil primate assemblage of Makapansgat may represent a primate taxon in the process of speciation. The variation in early hominins from this site is also pronounced (Arbor 2010). Much of this variation aligns with *Australopithecus africanus* while other aspects such as mandibular corpus size in MLD 40 and molar size and crown complexity in MLD 2 resemble *Paranthropus robustus*, and still other morphological features that are unique to the Makapansgat hominin assemblage (Aguirre 1970; Tobias 1980; Arbor 2010). Given the large mandibular corpus dimensions in both MLD 2 and MLD 40, they are likely to be males (although the former is a subadult). The males attributed to *Parapapio whitei*, MP 221 and MP 223, are similarly variable in M^1 dimensions (Tab. 2; Fig. 5). Cranial vault size differences between *Parapapio whitei* M3133 and MP 223 further argues for bimaturism among males (Tab. 1).

Diet and habitat of *Parapapio whitei*

The relatively larger size of *Parapapio whitei* (Delson et al. 2000) could imply a greater degree of terrestrial foraging than other taxa of the genus. At Sterkfontein Member 4, *Parapapio whitei* appears to have consumed more grasses and leaves than fruit compared *Pp. broomi* and *Pp. jonesi* (Benefit 1999). At Makapansgat, the diet of *Parapapio whitei* specimen MP 223 indicates hard and brittle foods or adhering grit were consumed, potentially from underground storage organs (Williams 2014) which could account for the partial C4 signal in the diet (Fourie et al. 2008). Indeed, the ecological processes that drove increasingly greater terrestriality in *Parapapio* could have also operated on *Australopithecus africanus*.

The two best preserved adult *Parapapio whitei* males, MP 221 and MP 223, of different sizes fall in between semi-terrestrial *Papio anubis* and tropical forest floor hard-object specialists typified by *Cercocebus agilis*. The differences between these two habitat preferences, tropical forest and woodlands, also seem to characterize reconstructions of the mosaic landscape of Pliocene Makapansgat, comprising wooded habitats interspersed by open areas with sources of water such as rivers in proximity (Reed et al. 2022). In this southern African habitat during the late Pliocene, *Parapapio whitei* and *Australopithecus africanus* likely competed for the same C3/C4 resources reflecting the mixed carbon isotopic signature characterizing both species (Codron et al. 2005). The two species probably fled from the same predators and contested vital resources such as sleeping sites (Benefit 1999). Although other primates such as *Theropithecus darti*, existed at Makapansgat, *Pp. whitei* is the largest species of the plentiful assortment of fossil cranial fragments attributed to *Parapapio* (Freedman 1976; Delson et al. 2000).

Its larger size placed it in more direct competition with early hominins such as *Australopithecus africanus*.

Conclusion

Parapapio whitei exhibits a relatively long M^1 that is as variable in minimum buccolingual dimensions and occlusal area as observed in *Papio anubis*. Yet this difference is between two males with relatively large canines, suggesting bimaturism or some other mechanism may account for the evolution of such intrasexual variability. The first maxillary molar outlines of the only two well-preserved individuals, that happen to be males, attributed to *Parapapio whitei* from Makapansgat present a range of variation that resembles what is observed in *Papio anubis*. With respect to both size and shape, MP 223 is more similar to *Papio anubis* while MP 221 resembles both *P. anubis* and *Cercocebus agilis*. However, squared Mahalanobis distances group the two fossils to *Parapapio whitei* and significantly differentiate them from all extant taxa suggesting these two males derive from the same taxon.

Parapapio whitei was the largest taxon of the genus in southern Africa and thus probably among the most terrestrial of the primate fauna living in the same habitats as *Australopithecus africanus. Parapapio whitei* and *Australopithecus africanus* probably competed for the same foods and other resources (Benefit 1999; Elton 2007). The difference in size between the two *Parapapio whitei* males presents the possibility that ecological variability, intrasexual competition or bimaturism could have evolved. The same processes could have also led to large intrasexual size distinctions in other semi-terrestrial primates, such as *Australopithecus africanus*.

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Conflict of interests

The authors have no conflicts of interest to declare. An employee may use, or permit the use of, his/her title in connection with an article published in a scientific or professional journal, provided that the title or position is accompanied by a reasonably prominent disclaimer stating that the views expressed in the article are the employee's and do not represent the Government.

Authors' contribution

ACK created the outlines and measurements of the dental casts and contributed to the writing and editing. FLW conceptualized the work; created the dental molds and casts and led the writing of the paper.

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Changes in body composition (muscle mass and adipose tissue) among adolescents aged 11–15 from Kraków during the COVID-19 pandemic

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ABSTRACT: Study aim: To assess changes in body composition, specifically focusing on muscle mass and adipose tissue, among adolescents aged 11–15 in Kraków during the COVID-19 pandemic.

Materials and Methods: Cross-sectional studies were conducted in four selected districts of the city of Kraków in the years 2020 and 2022 (before and after the COVID-19 pandemic). The study group included adolescents aged 11–15 years. The percentage of body fat (%BF) was measured using the bioimpedance method. Additionally, measurements of height, arm circumference, and skinfold thickness of triceps were taken using a skinfold calliper. The collected data were used to calculate the Corrected Arm Muscle Area (CAMA). The normality of the distribution of each feature was assessed using the Shapiro-Wilk test. Statistical analysis was performed to compare differences between groups using two-way ANOVA with Tukey's HSD post-hoc test or the Kruskal-Wallis test.

Results: Among girls, a decrease in muscle mass was observed in most age categories. The opposite trend was observed among boys, as an increase in muscle mass was observed in most of the age groups. Girls were characterized by a decrease in the %BF in all cohorts, while in boys, an increase in the %BF was observed among 11,13 and 15-year-olds. In most age groups, there was an increase in the average muscle mass and increase in the %BF depending on BMI (Body Mass Index) categories in both sexes.

Conclusions: This study found no notable variances in muscle mass and %BF within the examined group amid the COVID-19 pandemic. While certain outcomes indicated regression, possibly linked to reduced physical activity or prolonged sedentary periods, not all research findings exhibited decline. This could be attributed to online physical activity or enhanced dietary habits.

KEY WORDS: coronavirus, children, muscle mass, body fat

Original article

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Introduction

The disease caused by the SARS-CoV-2, has been recognized as one of the most serious pandemics in the world, significantly impacting the lifestyles of billions of children and young people (Karatzi et al. 2021; de Girolamo et al. 2022; Kutac et al. 2022; Musa et al. 2022). To prevent the spread of the coronavirus and the collapse of national healthcare systems, governments on all continents have imposed strict isolation measures on their citizens. The implemented precautionary measures included the closure of schools for a significant portion of 2020 at all levels of education, restrictions on travel and various forms of transportation, the closure of shops, restaurants, and all non-essential businesses where remote work was feasible, and the government mandated stay-at-home orders (Martinez-Ferren 2020; Damanti et al. 2021; Karatzi 2021; Musa et al. 2022). Unfortunately, some of the imposed restrictions, such as social distancing, closure of gyms and sports facilities, as well as limitations on outdoor activities have significantly impacted people's lifestyles, including overall health status, habits related to physical activity, diet, and other health-related behaviors (Lesser, Nienhuis 2020; Tison et al. 2020). Research has shown that during the pandemic unhealthy eating patterns have risen, including changes in the types of food consumed as well as changes in other eating habits that might have caused the loss of control over the caloric content of consumed food (Ammar et al. 2020). It has been suggested that the current lack of physical activity may lead to the formation of entrenched habits that persist after the SARS-CoV-2 crisis (Chen et al. 2020). The factors described above can also contribute to the occurrence of overweight, obesity, and a decline in physical fitness (Kovacs et al. 2021; Kutac et al. 2022). This is supported by the findings of an online survey conducted in Italy, showing that 49.6% of respondents did not change their diet, 46.1% of respondents reported eating more during the lockdown, and 19.5% gained weight. The survey revealed a notable increase in the consumption of "comfort food," particularly ice cream, chocolate, desserts (42.5%), as well as salty snacks (23.5%). Despite reduced access, 21.2% of participants increased their intake of fresh vegetables and fruits (Scarmozzino, Visioli; 2020). In turn, obesity was shown to be a significant public health issue that increases the risk of cardiometabolic problems such as cardiovascular diseases, diabetes, hypertension, and dyslipidemia, and it is correlated with an increased risk of COVID-19 complications (Krams et al. 2020; WOF 2020; Karatzi et al. 2021; Loza et al. 2022). Body composition analysis is one of the methods of assessing health status, nutritional status, and the impact of illness and dietary changes (Toomey et al. 2015; Kutac et al. 2022). Body composition measurement provides insights into the health status and lifestyle habits of young individuals. Body composition is influenced by genetic and exogenous factors, physical activity, nutrition, and overall health (Kutac et al. 2022). Studies among adolescents aged 11–18 have shown that the COVID-19 pandemic has significantly affected body composition. For example, there has been an increase in adipose tissue surface area and visceral adipose tissue, accompanied by a decrease in skeletal muscle mass. In addition, no significant changes in body weight and Body Mass Index (BMI) have been observed (Kutac et al. 2022).

Studies have shown that the pandemic may have contributed to an increase in the rate of weight gain among children and adolescents due to limited access to physical activity and extracurricular activities (Kovacs et al. 2021; Loza et al. 2022). Data collected worldwide have documented a significant decrease in physical activity, coupled with an increase in screen time during the SARS-CoV-2 virus-induced pandemic (Kovacs et al. 2021). Research on Austrian children aged 7–10 further highlights these trends, revealing a decline in cardiovascular-respiratory fitness alongside increases in BMI SDS values. The prevalence of overweight and obesity among these children rose from 15.0% to 21.2% when analysing the results before and after the COVID-19 pandemic (Jarnig et al. 2022). These data are supported by observational studies (n=8395) conducted in 10 European countries, which reported the exceeding of the daily screen time limit (>2 hours/day) among 69.5% of young individuals aged 6–18 during weekdays and 63.8% on weekends (Kovacs et al. 2021). Furthermore, among adolescents in Shanghai, an increase from 21.3% to 65.6% in physically inactive students was recorded (Xiang et al. 2020).

The aim of the study was to assess changes in body composition, specifically the proportion of muscle mass and adipose tissue, among adolescents aged 11–15 from Kraków during the COVID-19 pandemic.

Materials and methods

The adolescents analysed in this study were included in two series of cross-sectional studies conducted in 2020 and 2022, carried out in randomly selected primary schools located in four traditional districts (Śródmieście, Podgórze, Krowodrza, Nowa Huta) of Kraków.

The study group consisted of adolescents aged 11–15, who were divided by age and sex. The calendar age of the participants was calculated as the difference between the date of the study and the date of birth, expressed as a decimal fraction. The calendar age of the participants ranged from 10.50 to 15.49 years and served as the basis for classifying each participant into one of five age groups (e.g., adolescents aged 10.50–11.49 years were classified as 11-year-olds). The sample size included 1069 individuals in 2020 (557 girls, 512 boys) and 593 children in 2022 (288 girls, 305 boys) (Tab. 1).

Table 1. Number of children included the sample

Age category [yrs.]	2020		2022	
	Girls	Boys	Girls	Boys
11	170	115	94	72
12	107	110	78	79
13	87	115	54	77
14	93	79	44	46
15	100	93	18	31
Total	557	512	288	305
	1069		593	

The data from the 2022 survey series was compared with the results from the series in 2020. The study was conducted as recommended by the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008 and was approved by the Bioethics Committee of the Regional Medical Association in Kraków (5/KBL/OIL/2019, 66/KBL/OIL/2022). In addition, the study was conducted with the consent of school principals. The basis for the inclusion of the participant in the study was the written consent of the parent/legal guardian, as well as the oral consent of the subjects themselves before the study begin. Possible exclusion from participation was based on the lack of consent of the parent/legal guardian, participant or information about contraindications to the study.

In both studies, researchers have followed COVID-19 standard operating procedures.

All anthropometric measurements took place in the morning hours. The youth were dressed in gym clothes and were barefoot. The anthropometric data obtained in this study covered the winter-spring period (January–May) in 2022, which were analysed comparatively with the results of the "Child of Krakow 2020" project, conducted in the winter-spring period (January–March) in 2020, just before the year in which the COVID-19 pandemic began. Both studies collected data from the same schools.

Body height was measured using an anthropometer (GPM, Switzerland, with an accuracy of 1mm) according to Martin's technique. To measurement of the percentage of adipose tissue was conducted using the portable segmental body composition analyser (bioimpedance method BIA; Tanita, Japan, with an accuracy of 0.1%). The study was conducted according to a standard protocol for all participants. The measurements were taken in the morning hours, under similar temperature and humidity conditions.

A non-stretch anthropometric (with an accuracy of 5mm) tape was used to measure the circumference of the arm (cm). The arm circumference was measured at the midpoint of the humerus bone. The measurement of the triceps brachii skinfold (in mm) was taken at the midpoint of the arm, on the right side of the body, using a skinfold calliper (a constant pressure of 10g/mm^2 and an accuracy of 0.5mm). Both measurements were used to calculate the corrected arm muscle area (CAMA). Total body muscle mass (kg) was calculated from CAMA using a prediction equation:

Formula for women:

$$CAMA= [(MAC-(\pi \times TSF))^2/4\pi]-6,5$$

Formula for men:

$$CAMA= [(MAC-(\pi \times TSF))^2/4\pi]-10$$

TSF denotes triceps skinfold and MAC mid-arm circumference (cm) while the term 6.5 refers to the correction for bone area in females and 10 for men.

$$\text{Muscle mass (kg)} = H \times (0.0264+(0.0029 \times CAMA))$$

H- body height (cm)

Statistical analysis was performed using the Statistica software (version 13.3). To compare the differences between the 2020 and 2022 cohorts, two-way ANOVA and Kruskal-Wallis test were performed. According to the assumptions, the observable effect should reflect both waves of the pandemic.

Results

Girls

A decrease in muscle mass was observed among the examined girls in the majority of age groups, analysing the cohort from 2022 compared to peers examined in 2020 (the difference was statistically significant among 14-year-olds). However, among 11- and 12-year-old girls the opposite trend was observed, as muscle mass increased (Tab. 2).

Table 2. Mean and SD values of muscle mass and body fat in particular age categories and differences between cohorts

Age category [years]	Muscle mass [kg]					BF [%]				
	2020		2022		2022 vs 2020	2020		2022		2022 vs 2020
	$\bar{x}$	SD	$\bar{x}$	SD		$\bar{x}$	SD	$\bar{x}$	SD	
Girls										
11	14.50	3.76	14.72	3.67	0.22	19.96	6.35	19.45	6.26	-0.51
12	16.82	3.78	17.02	4.82	0.20	21.40	6.60	20.91	6.69	-0.49
13	18.11	3.83	17.62	3.51	-0.49	21.79	5.96	21.45	5.94	-0.34
14	21.03	4.40	18.55	4.75	-2.48**	24.11	6.53	21.00	6.52	-3.11**
15	21.60	4.57	20.49	3.83	-1.11	24.27	7.44	23.19	6.07	-1.08
Boys										
11	14.97	3.68	15.93	4.87	0.96	16.88	6.63	18.07	7.03	1.19
12	17.91	5.05	17.37	4.74	-0.54	16.48	6.91	16.36	5.85	-0.12
13	20.09	4.66	19.84	5.28	-0.25	14.90	7.03	14.94	6.49	0.04
14	22.68	6.20	23.74	5.94	1.06	13.27	6.07	12.75	5.07	-0.52
15	27.11	6.57	27.50	6.47	0.39	13.01	6.75	14.24	5.46	1.23

**- statistically significant $p<0.01$

Furthermore, the analysis of the %BF in girls examined in 2022 compared to 2020 showed a general decrease in all age categories. There was a statistically significant difference among 14-year-olds (Tab. 2).

Figure 1 illustrates the average percentage of body fat according to BMI categories. Among girls, an increase in the occurrence of both underweight and overweight was observed. In addition, an increase in the average values for all BMI categories was observed. However, an opposite trend was observed in the case of normal body weight (Fig. 1).

Analogous values were observed when analysing the results of average muscle mass in the context of BMI categories (Fig. 2).

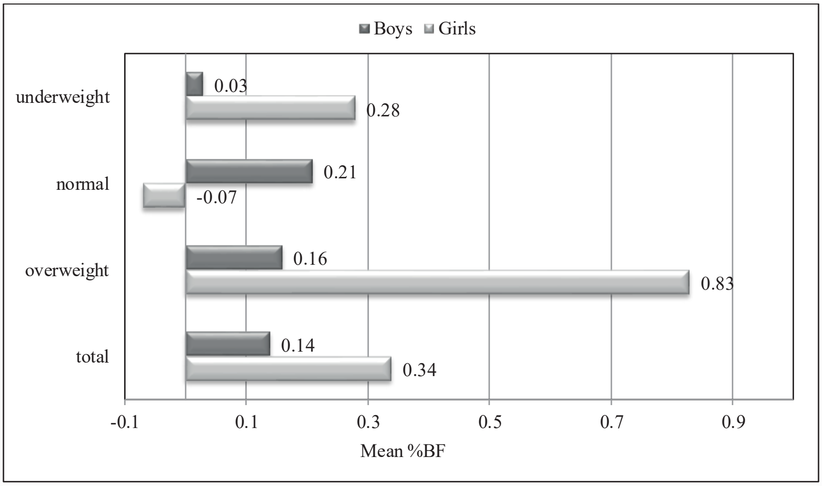

Figure 1. Mean ($\overline{x}$) of % Body Fat for the Total (girls and boys)

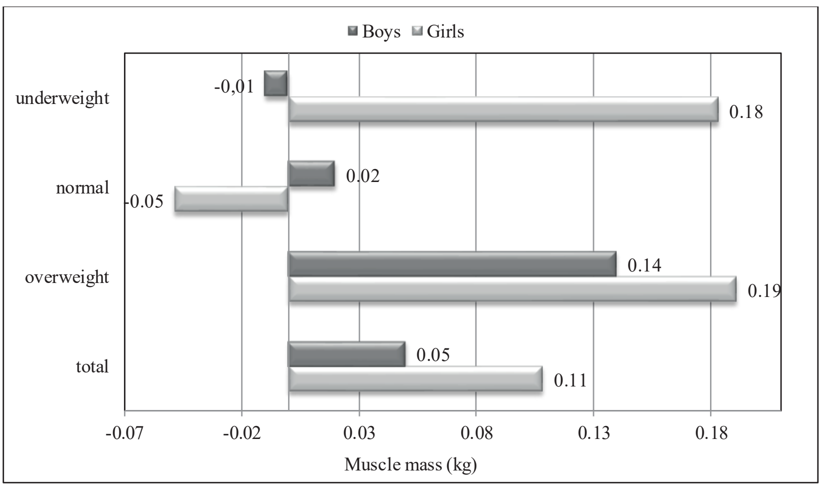

Figure 2. Mean ($\overline{x}$) of muscle mass for the Total (girls and boys)

Boys

In boys aged 11, 13, and 15, an increase in muscle mass was observed, analysing the results from 2022 compared to boys examined in 2020. Among the other groups (12 and 14 years), a decrease in muscle mass was observed but without significant differences (Tab. 2). In addition, an increase in %BF was observed in most age groups, except for 12 and 14-year-old boys who exhibited a decreasing pattern (Tab. 2). There was an increase in all average body fat percentage values in different BMI categories (Fig. 1).

Moreover, Figure 2 depicts the results of the average muscle mass in various BMI categories. An increase in both normal body weight and overweight was observed. Furthermore, there was an increase in the average values for all BMI categories. Only a different trend was observed in the underweight category (Fig. 2).

Discussion

Body composition

The high prevalence of obesity has reached the dimensions of a pandemic, especially in developed countries in the West. Obesity in children and adolescents poses a significant health threat, and is associated with insulin resistance, risk factors for cardiovascular diseases, type 2 diabetes, cancers, and increased mortality (Trang et al. 2019; Stierman et al. 2021; Brambilla et al. 2022). According to the assessment of the World Health Organization (WHO), obesity, as a non-communicable disease, is a key risk factor for severe illness resulting from the SARS-CoV-2, which was operating as a global pandemic characterized by severe pneumonia and a significant level of mortality and morbidity (Malavazos et al. 2020, WHO 2020). Although government-imposed restrictions, such as social distancing, home isolation, limitations on travel and gatherings were necessary non-pharmaceutical interventions to control the spread of the SARS-CoV-2, they significantly impacted the health and behaviours of society, as well as the daily routines of people worldwide (Wang et al. 2020; Jeong et al. 2023). Remaining inactive has become more accessible than ever before. As a result of these restrictions, there has been a reduction in physical activity, an increase in sedentary time, and changes in dietary habits (Kirwan et al. 2020). According to a study by Rúa-Alonso et al. (2022), the COVID-19 pandemic-induced social restrictions may have had a negative impact on the body composition of children and adolescents, resulting in increased rates of obesity, as well as a reduction in muscle strength. Hence, it is important to monitor body composition among the young population, reflecting their behaviour and potentially impacting their current health status (Hernández-Ortega, Osuna-Padilla 2020). To address these issues, this study was conducted to assess changes in body composition, with particular emphasis on the proportion of muscle mass and adipose tissue, among adolescents aged 11–15 in Kraków during the COVID-19 pandemic. The results of this study suggest that restrictions related to limiting the spread of the SARS-CoV-2 had contributed to changes in muscle mass and %BF among adolescents in Kraków. Among girls, a decrease in muscle mass was observed among most of the age groups. Conversely, boys showed the opposite trend, as an increase in muscle mass was observed in most of the age categories. Girls exhibited a decrease in the percentage of body fat in all age categories, whereas boys showed an increase in the percentage of body fat in most of the age groups. In addition, an increase in the average muscle mass was observed in most the age groups, depending on the BMI category in both sexes. Furthermore, an increase in body fat was observed in both girls and boys across all BMI categories.

Physical activity

Similar results were observed in young basketball players, as during the quarantine period caused by the SARS-CoV-2 virus, higher values of body fat and lower percentages of total muscle mass were noted. In addition, a lower level of explosive strength in the lower limbs was observed among the subjects, which was accompanied by lower aerobic endurance. One study suggests that the interruption of the training process and a decrease in physical activity due to the restrictions imposed during the coronavirus pandemic had a negative impact on the condition of young basketball players (Pelemiš et al. 2023). A study conducted in the Kraków population also showed that the level of physical fitness in children during the pandemic worsened markedly (Artymiak et al. 2024). For proper functioning and maintenance of muscle mass during the developmental period, it is crucial to achieve the level of daily physical activity recommended by the WHO, which is at least 60 minutes of moderate- or vigorous-intensity activity per day, covering the age group from 5 to 17 years. Unfortunately, over 80% of teenagers worldwide are not sufficiently active physically (Kutac et al. 2020; Mondaca et al. 2023; WHO 2022). A survey-based study conducted internationally with participants primarily from Asia, Africa, and Europe reported that, amidst the lockdown imposed by the coronavirus pandemic, there was a 33.5% reduction in the amount of time dedicated to daily physical activity. In addition, the study reported an uptick in sedentary behaviour, amounting to an increase of 3 hours per day (Ammar et al. 2020). Similar studies were conducted among children and adolescents aged 6–18 living in Italy showing a decrease in sports physical activity by 2.3 hours per day. Furthermore, an extension of sedentary time and screen time by 4.85 hours/day was observed (Kirwan et al. 2020). These results correspond to findings of this study, as a decrease in physical activity was observed, which may be associated with a reduction in muscle mass among surveyed girls in all age categories. It could be suggested that the introduced isolation measures due to the SARS-CoV-2 contributed to a decrease in physical activity, which may be associated with an increase in sedentary lifestyle and screen time.

Dietary habits

Longer time spent in front of the screen may adversely affect the younger generation, both in terms of sleep quality and the loss of muscle mass associated with stress and sleep restriction (Kirwan et al. 2020; Ito et al. 2022). Recommendations regarding 24-hour movement suggest limiting screen time to less than two hours per day and ensuring an adequate amount of sleep, depending on the age group. Studies on Swedish youth indicated that after the pandemic, the time dedicated to sleep has shortened, while screen time has lengthened (Helgadóttiret al. 2023). Shortened sleep duration may also affect appetite and eating habits, increasing the consumption of snacks, fast foods, and sugary drinks (Morselli et al. 2010; Chang et al. 2021). This, in turn, could lead to unfavourable changes in the relationship between calories consumed and expended, influencing fat storage and BMI increase. It is worth noting that youth showing normal body fat content typically exhibits better dietary patterns compared to their overweight and obese peer. Childhood and early adolescence are crucial for developing physical skills that form the foundation of a healthy lifestyle throughout life (Maltoni et al. 2021; Kutac et al. 2022; Syukrina et al. 2023). Moreover, a study conducted in Italy using online questionnaires showed an improvement in eating habits exhibited by a higher consumption of fruits, vegetables, and legumes during the isolation caused by the coronavirus pandemic (Grant et al. 2021). Similarly, cross-sectional studies involving the adult population in Spain revealed a positive impact of isolation caused by SARS-CoV-2 on dietary habits and physical activity (López-Bueno et al. 2020).

This study has several limitations. Firstly, due to the prolonged isolation caused by the COVID-19 pandemic, a limited number of students from the oldest age group consented to participate in the study. Secondly, the long period of break in school activities caused by the pandemic could have caused discomfort for some students with higher body mass during anthropometric measurements. This phenomenon could have affected the representativeness of the sample in the context of body composition.

In this cross-sectional study, minor changes in muscle mass and body %BF among the examined population during the COVID-19 pandemic. The results of this study may reflect changes in physical activity or increased time spent in a sitting position. On the other hand, not all results showed a decreasing trend, which may be attributed to participation in online or outdoor activities and the improvement of diet quality during the isolation associated with the coronavirus pandemic. In addition, minor changes in the results may be associated with the process of easing restrictions, which favours increased physical activity and outdoor time among children and adolescents, including the possibility of using outdoor gyms. Therefore, the results of this study suggest that promoting physical activity and healthy eating habits among young people is important to encourage them to maintain a healthy lifestyle and prevent potential health problems, especially in extraordinary situations.

Authors' contributions

PA, MŻ, ŁK have made substantial contributions to conception and design. PA, MŻ, MŻ contributed to the acquisition of data. PA, MŻ prepared analysis and interpretation of data. PA, MŻ have been involved in drafting the manuscript and revising it critically for important intellectual content. PA, MŻ, ŁK have given final approval for the version to be published.

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Conflict of interest

The authors have no conflicts of interest to declare.

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How do age and sex influence pain threshold and tolerance among Santal tribal people living in West Bengal, India?

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Abstract: The perception of pain, encompassing pain threshold and tolerance levels, is a complex phenomenon influenced by biological, psychological, and sociocultural factors. Notably, age and sex have consistently emerged as pivotal determinants in modulating pain perception. The study aimed to examine age and sex differences in pain threshold and tolerance levels. Furthermore, it delved into exploring whether age-related differences in pain threshold and tolerance levels vary between males and females. This study incorporated 484 healthy Santal tribal individuals aged 18–88 years (male 203 and female 281) living in Howrah and Purba Bardhaman Districts of West Bengal State, India, who reported no chronic or significant pain at the time of data collection. Pain threshold and tolerance levels were assessed using a digital algometer. Results of two-way ANOVA revealed significant main effects of age and sex on every pain threshold and tolerance level assessed in this study, indicating that older individuals had lower pain threshold and tolerance levels than younger ones. Males demonstrated greater levels of pain threshold and tolerance relative to females. Age and sex showed a significant interaction effect on pain tolerance levels, but not on pain threshold levels demonstrating the age-associated declining trend in pain threshold levels was consistent for either sex; however, such a tendency in pain tolerance levels was more pronounced in men. These findings highlight the importance of considering age and sex factors when assessing pain perception.

Key words: pain threshold, pain tolerance, age, sex, interaction effect

Original article
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Introduction

In 2020, the International Association for the Study of Pain (IASP) revised the definition of pain to describe it as 'an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage' (Stevens 2021). Pain is one of the vital signs that signal the body about potential threats. Pain presents a dual nature, providing the body with protection (Belfer 2013), while also engendering discomfort, interfering with daily activities, and potentially leading to dependency and institutionalization in severe cases (Yağci et al. 2014). These facets underscore the multidimensional nature of pain. Prompt responses to pain not only safeguard the body against further damage but also contribute to sustaining a healthy existence (Lorusso et al. 2018). Therefore, the perception of pain emerges as a crucial aspect for human survival and overall well-being (Swift 2018).

Pain perception, encompassing pain threshold and tolerance levels, along with the management of pain, represent foundational aspects of human well-being and healthcare (DiMatteo and Martin 2002). The minimal stimulus required to elicit a painful sensation is denoted as the pain threshold (Schmitz et al. 2013), while the maximum amount of pain an individual can endure is defined as the pain tolerance (Cimpean and David 2019). Gaining insights into the factors that influence an individual's pain threshold and tolerance is of paramount importance for devising successful approaches to pain management (Roy et al. 2013).

Pain perception is a multifaceted phenomenon influenced by a myriad of biological, psychological, and sociocultural factors (Bartley and Fillingim 2013; Shipton 2013; Ferreira et al. 2015). Among these, age has consistently emerged as a critical factor in shaping an individual's response to pain (Yezierski 2012). Generally, age-related changes in the nervous system, such as alterations in nerve conduction velocity and decreased pain modulation, have been proposed as contributors to variations in pain sensitivity across the lifespan (Yezierski 2012). While it is recognized that older individuals might experience reduced pain, comprehending exactly how age affects pain thresholds and tolerance remains significant in this context. For example, the frequent absence of pain in older patients with conditions like myocardial infarction or peptic ulcer disease suggests potential alterations in pain thresholds (Moore and Clinch 2004). Despite these observations, the older adults reported experiencing frequent pain. Chronic pain becomes more prevalent as individuals age (Domenichiello and Ramsden 2019), affecting more than half of older adults residing in the community (Gibson 2003). This incident emphasizes the complex nature of age-related changes in pain. Moreover, the findings of the previous experimental studies on age-associated changes in pain threshold and tolerance are inconsistent and equivocal (Pickering et al. 2002; Lautenbacher et al. 2005; Cole et al. 2010; Petrini et al. 2015). For that reason, the precise nature and extent of these age-related alterations and their relevance for pain management remain areas of active investigation.

Sex, too, has been implicated in the modulation of pain perception. Similar to age, investigations into sex-related differences in pain sensitivity have yielded a multitude of diverse findings (Racine et al. 2012). Several experimental investigations have documented variations in pain sensitivity across sexes (Pelfort et al. 2015; Cámara et al. 2020), suggesting that hormonal, genetic, and psychosocial factors may contribute to these disparities (El Tumi and Tashani 2017). However, it is well documented that females tend to report a higher prevalence and intensity of pain compared to males (Lue et al. 2018; Overstreet et al. 2023). This sex-related variation in pain perception has prompted intriguing arguments over whether they are biologically determined versus socially constructed and if they should inform gender-specific approaches to pain management (Gazerani et al. 2021).

The exploration of age- and sex-related differences in pain perception has garnered significant research attention, with numerous attempts to elucidate these complexities. However, whether age-related alterations in pain perception vary between males and females remains largely unexplored. Females undergo pronounced hormonal fluctuations across their life span, with hormonal levels rising and falling cyclically during the menstrual cycle. These fluctuations have been associated with varying levels of pain sensitivity (Fillingim et al. 1997). Additionally, the occurrence of menopause in females leads to a substantial reduction in estrogen levels, with a lesser impact on progesterone levels (Burger et al. 2007). Notably, research has shown that estrogen has analgesic properties, and the increased pain sensitivity during menopause is linked to a drop in estrogen levels (Nikolov and Petkova 2010). Consequently, because of this increased pain sensitivity, women may be more susceptible to conditions like chronic pain, headaches, and joint pain (Meriggiola et al. 2012). In contrast, males do not experience analogous fluctuations in their primary sex hormone, testosterone, throughout different life stages, which is considered to play an anti-nociceptive role (Vincent and Tracey 2008). Besides, a recent study uncovered variations in age-related alterations in the endogenous opioid system across genders. Specifically, females demonstrated a decline in the functionality of the descending pain modulatory system (a network of neural pathways in the central nervous system that regulates pain perception), while males exhibited an upward trend in the activity of this system (Failla et al. 2024). Therefore, it is plausible to anticipate that age-related changes in pain threshold and tolerance levels may not exhibit a uniform pattern between males and females.

In India, there is a scarcity of research among the tribal communities concerning pain sensitivity and its associated factors, specifically age and sex. Moreover, the distinction between males and females extends beyond biological factors to include social and cultural dimensions. The term "tribe" typically refers to an ethnic group that is geographically isolated or semi-isolated, associated with a specific territory, and characterized by unique social, economic, and cultural traditions and practices (Singh and Singh 2017). Tribal communities in India exhibit distinct cultural characteristics, setting them apart from the rest of the general population (Boro and Saikia 2020). It remains unknown how these two factors influence their pain threshold and tolerance levels. Given the clinical relevance of age and sex as potential modifiers of pain perception, the present study intended to determine whether there exists any difference in the pain threshold and tolerance level across different age groups and between two sexes belonging to the Santal tribal community. Furthermore, the investigation aimed to determine whether the age-associated difference in pain threshold and tolerance levels exhibits variations between males and females. In other words, the study investigated the main effects as well as the interaction effects of age and sex among the Santal tribal people residing in West Bengal, India.

Materials and methods

The data utilized in this study were derived from a large community-based study centered on exploring different aspects of pain, such as pain sensitivity, musculoskeletal pain-related cognitions, and treatment-seeking behavior for musculoskeletal pain (Santra et al. 2024). Participants were recruited from the Santal tribal community residing in two districts, namely Howrah and Purba Bardhaman, within the state of West Bengal, India. Within each district, settlements of Santals were chosen based on their higher population density or numerical representation in the area. The selection of study participants from such eight settlements followed a two-step process. In the first step, a demographic survey was conducted through door-to-door visits, during which socio-demographic information of all adult household members was collected. When an adult member was absent during this survey, the head of the household or any senior member of the same household provided the details. While collecting the details, the objectives and purpose of the study were explained to every household head and adult individual in the households, and verbal consent was obtained. This step covered 343 households and identified 1155 eligible participants (546 males and 609 females) who were aged 18–88 years and permanent residents of the selected areas. In the second step, the collection of all the required data began after compiling a comprehensive list of eligible participants. Prior to assessing pain threshold and tolerance, the procedure of testing with the instrument was clearly demonstrated to every individual we approached, and written consent was obtained. At this phase of data collection, 390 individuals were unavailable, and 67 individuals withdrew their participation. 685 individuals agreed to participate in the study and completed the survey, providing the required data. They also consented to the measurement of pain threshold and tolerance levels. However, for the purpose of the present study, we fixed some inclusion criteria: (1) currently experiencing no pain; (2) free from chronic illnesses like diabetes, hypertension, chronic pain, etc.; and (3) no disability or existing injury in the body at the time of the survey. Following these criteria, 13 individuals were eliminated for having an injury or disability. Further, to maintain homogeneity, we dropped 201 individuals with any known chronic conditions or who were afflicted with pain during the time of the survey. Eventually, a total of 484 individuals, including 203 males and 281 females, were incorporated into the present study.

Socio-demographic data that included participants' present age (in years), sex (categories: male and female), educational attainment, marital status (categories: unmarried, married, and widowed/separated/divorced), working status (categories: working and non-working), household characteristics (e.g., house type, toilet facilities, and other related factors), and possession of household assets (e.g., television, refrigerator, car, and other similar aspects) (IIPS and ICF 2017) were obtained using a structured questionnaire. Age was further subdivided into three age groups viz. young (18–34 years), middle-aged (35–49 years), and old (50 years and older) based on an adaptation of a prior study that utilized finer age categories (e.g., 18–34, 35–49, 50–64, 65–74, and 75 and older) (Elgaddal et al. 2024). However, in the present study, the sample sizes in the older age groups were relatively small, particularly in individuals aged 65 years and above. To ensure sufficient sample sizes within each group and maintain statistical power for the analyses, the age categories were consolidated into three broader groups. Education level was further categorized into five levels: having no formal education, primary (had education between classes 1–4), secondary (had education between classes 5 and 10), higher secondary (had education between classes 11 and 12), and graduation and above (had education up to graduation level and beyond). Following the Demographic and Health Surveys (the DHS program), a wealth index was constructed for each participant based on their respective household assets and characteristics using a principal component analysis with varimax rotation (Rutstein 2008). The first principal component (PC) (eigenvalue > 1), which was able to explain the largest proportion of the total variance (33.22%), was taken to represent the wealth index. Further, the factor scores of the first PC were divided into three equal segments. Participants scoring below the 33rd percentile were categorized in the lower stratum; those scoring between the 34th and 66th percentile were placed in the middle stratum; and those scoring above the 66th percentile were assigned to the upper stratum of socioeconomic class.

A digital pressure algometer (Model: ALGO-DS, Orchid Scientific, India) was used to measure the pain threshold and tolerance levels of the participants. The instrument has a 1 cm^2 rounded, blunt metal tip. Pain threshold levels were assessed on eight muscles bilaterally, including the right extensor carpi radialis, right biceps brachii, right triceps brachii, right upper trapezius, left extensor carpi radialis, left biceps brachii, left triceps brachii, and left upper trapezius muscles (Walton et al. 2011; Duan et al. 2014; Georgoudis et al. 2014). The participants were asked to sit comfortably on a chair with a straight back and relaxed arms, and the instrument was placed perpendicularly with its tip on the muscle points. After holding the instrument properly, pressure was gradually increased at a constant rate. They were asked to say 'stop' or 'pain' when they sensed the applied pressure as painful for the first time. The instrument was removed immediately from the muscle point, and the value (in kg) was recorded as the pain threshold level. The same protocol was followed for every threshold measurement. The pain tolerance level was measured at only one muscle point (*flexor carpi radialis*) on the right forearm. While measuring it, the participants were asked to report 'stop' or 'pain' when they were no longer able to withstand the pain, and the value was (in kg) taken as their pain tolerance level. Every measurement was taken with an interval of approximately two minutes.

SPSS version 24.0 (IBM Corporation, Armonk, NY, USA) was utilized for statistical analyses. Descriptive statistics (frequency and percentage, and mean and standard deviation) were employed to summarize the socio-demographic and pain threshold and tolerance data. χ^2 test was performed to compare categorical variables. Two-way ANOVA was implemented to evaluate both the main effects and interaction effects of age and sex on every pain threshold and tolerance level, considering age and sex as independent variables and the pain measurements as dependent variables. Further, post hoc pairwise comparisons with Bonferroni adjustments were performed to compare the mean difference across groups upon receiving significant main effects or interaction effects. Partial Eta squared values (denoted as η^2) represent the effect size, i.e., the magnitude of variability in dependent variables explained by independent variability. Partial η^2 values of 0.01, 0.06, and 0.14 indicate low, medium, and large effects, respectively (Cohen 1988). A p value of ≤ 0.05 was considered statistically significant.

Results

Table 1 shows the socio-demographic characteristics of the study participants. A significantly higher proportion of female participants belonged to the "middle-aged" group, whereas the distribution of males across the three age categories was quite similar. The majority of the participants were married, but the frequency of widowed/separated/divorced individuals was found to be considerably greater in females. Notably, a significant difference existed in educational attainment between the sexes, with nearly 30% of the males having completed their education up to the graduation level or above, while a substantial number of females lacked formal education. Regarding working status, although over half of the males and females were working, a significantly greater number of males were found to be employed when compared with the females. No significant difference was noted in the distribution of participants based on their respective wealth indexes.

Table 1. Socio-demographic characteristics of the study participants

Characteristics	Male	Female	χ^2 value (p value)
	n (%)	n (%)	
Age groups			
Young (18–34 years)	66 (32.51)	98 (34.88)	6.18 (0.05)
Middle-aged (35–49 years)	66 (32.51)	113 (40.21)	
Old (50 years and older)	71 (34.98)	70 (24.91)	
Marital status			
Unmarried	41 (20.20)	26 (9.25)	29.03 (<0.001)
Married	158 (77.83)	216 (76.87)	
Widowed/Separated/Divorced	4 (1.97)	39 (13.88)	
Educational attainment			
No formal education	36 (17.73)	99 (35.23)	25.44 (<0.001)
Primary	26 (12.81)	29 (10.32)	

Characteristics	Male	Female	χ^2 value (p value)
	n (%)	n (%)	
Educational attainment (cont.)			
Secondary	50 (24.63)	78 (27.76)	
Higher secondary	34 (16.75)	30 (10.68)	
Graduation and above	57 (28.08)	45 (16.01)	
Working status			
Non-working	59 (29.06)	125 (44.48)	11.89 (<0.001)
Working	144 (70.94)	156 (55.52)	
Wealth index			
Lower	67 (33.00)	104 (37.01)	0.88 (0.65)
Middle	69 (33.99)	92 (32.74)	
Upper	67 (33.00)	85 (30.25)	

The descriptive statistics of pain threshold and tolerance levels measured on different muscle points of the body are presented in Table 2. It shows that males had relatively higher mean values for every threshold and tolerance level than those of the females.

Table 2. Sex wise distribution of pain threshold and tolerance levels

Muscle points	Male	Female
	Mean (SD)	Mean (SD)
For pain threshold (kg)		
Right extensor carpi radialis	3.62 (1.22)	2.73 (0.88)
Right biceps brachii	3.17 (1.10)	2.47 (0.88)
Right triceps brachii	3.87 (1.33)	2.79 (0.91)
Right upper trapezius	3.84 (1.44)	2.90 (0.91)
Left extensor carpi radialis	3.40 (1.34)	2.61 (0.82)
Left biceps brachii	3.08 (1.12)	2.52 (0.86)
Left triceps brachii	3.57 (1.32)	2.65 (0.87)
Left upper trapezius	3.68 (1.52)	2.80 (0.89)
For pain tolerance (kg)		
Right flexor carpi radialis	9.67 (3.22)	7.10 (1.81)

The age group-wise distribution of pain threshold and tolerance values is portrayed in Table 3. Both the pain threshold and tolerance values show inconsistent values across all age groups.

Table 3. Age group wise distribution of pain threshold and tolerance level

Muscle points	Young	Middle-aged	Old
	Mean (SD)	Mean (SD)	Mean (SD)
For pain threshold (kg)			
Right extensor carpi radialis	3.07 (1.12)	3.19 (1.15)	3.02 (1.09)
Right biceps brachii	2.79 (0.99)	2.86 (1.12)	2.62 (0.97)
Right triceps brachii	3.19 (1.17)	3.35 (1.27)	3.17 (1.22)
Right upper trapezius	3.13 (1.01)	3.43 (1.28)	3.30 (1.44)
Left extensor carpi radialis	2.93 (1.10)	3.00 (1.26)	2.88 (1.02)
Left biceps brachii	2.80 (1.02)	2.81 (1.03)	2.63 (0.98)
Left triceps brachii	3.05 (1.16)	3.11 (1.25)	2.94 (1.08)
Left upper trapezius	3.03 (1.04)	3.28 (1.32)	3.19 (1.44)
For pain tolerance (kg)			
Right flexor carpi radialis	8.75 (2.96)	8.36 (2.92)	7.28 (2.18)

The results of two-way ANOVA are shown in Table 4. It is observed that except for two values taken on the left extensor carpi radialis and left upper trapezius muscle points, age had a significant main effect on all the pain threshold levels measured on the right extensor carpi radialis, right biceps brachii, right triceps brachii, right upper trapezius, left biceps brachii, and left triceps brachii muscle points. However, the partial η^2 values exhibited a small effect of age on pain threshold levels. In the case of pain tolerance level, age also exhibited a significant main effect with a medium effect size. Regarding the main effects of sex, at every threshold and tolerance level, sex was found to have a significant main effect. Here, partial η^2 indicated a medium-to-high effect of sex. Furthermore, the table indicates that there was a significant interaction effect of age and sex on the given pain tolerance level but not on any of the pain threshold levels. Overall, the findings suggest that males had significantly higher pain threshold and tolerance levels compared to females; pain tolerance as well as the majority of the pain threshold values considered in this study differed across the age groups; and the age-related changes in the pain tolerance levels varied between the sexes.

Table 4. Results of two-way ANOVA

Source	Muscle points	Type III sum of squares	df	F	*p* value	Partial η^2
Main effect of age	*For pain threshold*					
	Right extensor carpi radialis	6.30	2	2.96	0.05	0.01
	Right biceps brachii	9.08	2	4.79	0.01	0.02
	Right triceps brachii	8.17	2	3.38	0.03	0.01
	Right upper trapezius	10.56	2	3.97	0.02	0.02

Source	Muscle points	Type III sum of squares	df	F	*p* value	Partial η^2
Main effect of age	Left extensor carpi radialis	3.83	2	1.68	0.19	0.01
	Left biceps brachii	6.08	2	3.20	0.04	0.01
	Left triceps brachii	7.04	2	3.02	0.05	0.01
	Left upper trapezius	7.92	2	2.79	0.06	0.01
	For pain tolerance					
	Right flexor carpi radialis	277.61	2	25.08	<0.001	0.09
Main effect of sex	*For pain threshold*					
	Right extensor carpi radialis	96.92	1	91.15	<0.001	0.16
	Right biceps brachii	61.43	1	64.90	<0.001	0.12
	Right triceps brachii	143.02	1	118.26	<0.001	0.20
	Right upper trapezius	108.38	1	81.56	<0.001	0.15
	Left extensor carpi radialis	74.78	1	65.46	<0.001	0.12
	Left biceps brachii	38.53	1	40.56	<0.001	0.08
	Left triceps brachii	104.64	1	89.86	<0.001	0.16
	Left upper trapezius	94.94	1	66.74	<0.001	0.12
	For pain tolerance					
	Right flexor carpi radialis	821.57	1	148.45	<0.001	0.24
Interaction effects of age and sex	*For pain threshold*					
	Right extensor carpi radialis	0.44	2	0.21	0.81	0.00
	Right biceps brachii	1.15	2	0.61	0.55	0.00
	Right triceps brachii	0.00	2	0.00	1.00	0.00
	Right upper trapezius	3.03	2	1.14	0.32	0.00
	Left extensor carpi radialis	2.66	2	1.16	0.31	0.00
	Left biceps brachii	0.14	2	0.07	0.93	0.00
	Left triceps brachii	0.12	2	0.05	0.95	0.00
	Left upper trapezius	1.41	2	0.50	0.61	0.00
	For pain tolerance					
	Right flexor carpi radialis	101.68	2	9.19	<0.001	0.04

The pairwise comparison of pain threshold and tolerance levels with Bonferroni adjustment between different age groups (presented in Table 5) illustrates that the old group reported the lowest pain threshold values compared to the young and middle-aged groups. Moreover, Figure 1 demonstrates that the middle-aged individuals experienced increased pain threshold levels, and the values declined thereafter. The trend was similar for both sexes. Additionally, the findings highlight a declining trend in the pain tolerance level with the advancement of age.

Table 5. Pairwise comparisons of pain threshold and tolerance levels between age groups

Muscle points	Age groups (I)	Age groups (J)	Mean difference (I-J)	SE	*p* value	95% confidence interval Lower bound	95% confidence interval Upper bound
For pain threshold							
Right extensor carpi radialis	Young	Middle-aged	-0.14	0.11	0.71	-0.41	0.14
	Young	Old	0.15	0.12	0.62	-0.14	0.44
	Middle-aged	Old	0.29	0.12	0.05	0.00	0.57
Right biceps brachii	Young	Middle-aged	-0.07	0.11	1.00	-0.33	0.19
	Young	Old	0.26	0.11	0.07	-0.01	0.53
	Middle-aged	Old	0.33	0.11	0.01	0.06	0.60
Right triceps brachii	Young	Middle-aged	-0.20	0.12	0.32	-0.49	0.10
	Young	Old	0.12	0.13	0.99	-0.18	0.43
	Middle-aged	Old	0.32	0.13	0.03	0.02	0.62
Right upper trapezius	Young	Middle-aged	-0.35	0.13	0.02	-0.65	-0.04
	Young	Old	-0.08	0.13	1.00	-0.41	0.24
	Middle-aged	Old	0.26	0.13	0.14	-0.06	0.58
Left biceps brachii	Young	Middle-aged	-0.04	0.11	1.00	-0.30	0.22
	Young	Old	0.23	0.11	0.13	-0.04	0.50
	Middle-aged	Old	0.26	0.11	0.05	0.00	0.53
Left triceps brachii	Young	Middle-aged	-0.10	0.12	1.00	-0.39	0.19
	Young	Old	0.20	0.13	0.33	-0.10	0.50
	Middle-aged	Old	0.30	0.12	0.05	0.00	0.60
For pain tolerance							
Right flexor carpi radialis	Young	Middle-aged	0.32	0.26	0.67	-0.31	0.95
	Young	Old	1.82	0.27	<0.001	1.16	2.47
	Middle-aged	Old	1.50	0.27	<0.001	0.85	2.15

Table 6. Pairwise comparisons of pain tolerance levels between age groups for males and females

Age groups (I)	Age groups (J)	Mean difference (I-J)	SE	*p* value	95% confidence interval Lower Bound	95% confidence interval Upper Bound
Male						
Young	Middle-aged	0.50	0.41	0.68	-0.49	1.48
Young	Old	2.92	0.40	<0.001	1.95	3.88
Middle-aged	Old	2.42	0.40	<0.001	1.45	3.38
Female						

Age groups		Mean difference	SE	*p* value	95% confidence interval	
(I)	(J)				Lower bound	Upper bound
Young	Middle-aged	0.14	0.32	1.00	-0.64	0.92
Young	Old	0.72	0.37	0.15	-0.16	1.61
Middle-aged	Old	0.58	0.36	0.31	-0.28	1.44

Table 6 and Figure 1 show that males and females exhibited differences in the age-associated changes in pain tolerance levels. The males had a sharp decline in pain tolerance level along with increasing age. The females also had a similar trend but failed to show a statistically significant difference in mean pain tolerance values across various age groups.

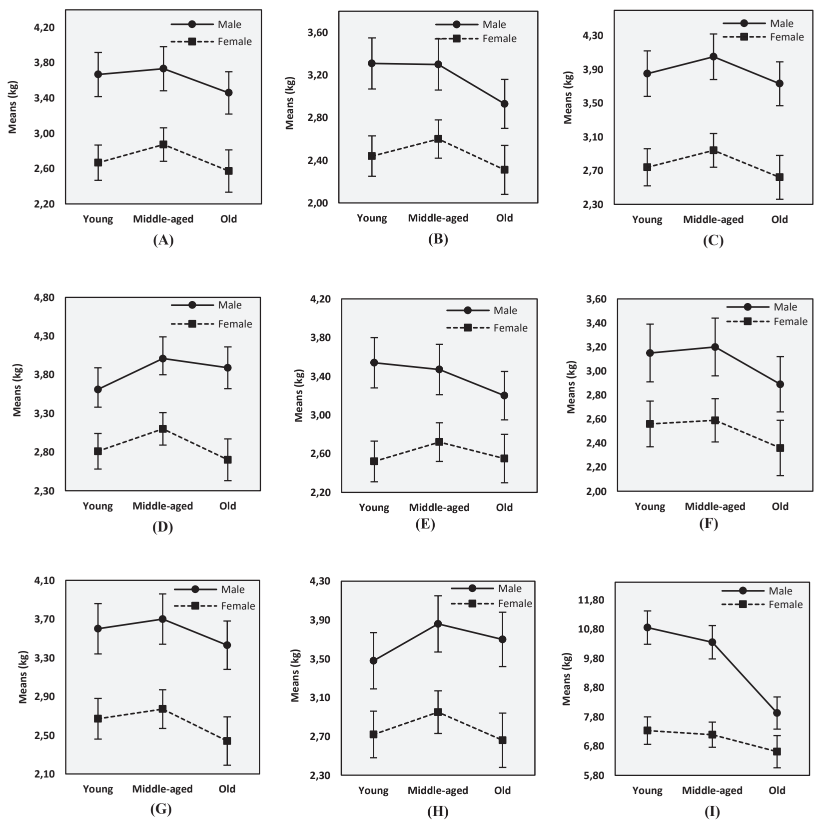

Figure 1. Results of two-way ANOVA with means and 95% confidence intervals for the pain threshold and tolerance levels taken on different muscle points among males and females of different age groups (A) Pain threshold on right extensor carpi radialis, (B) Pain threshold on right biceps brachii, (C) Pain threshold on right triceps brachii, (D) Pain threshold on right upper trapezius, (E) Pain threshold on left extensor carpi radialis, (F) Pain threshold on left biceps brachii, (G) Pain threshold on left triceps brachii, (H) Pain threshold on left upper trapezius, (I) Pain tolerance on right flexor carpi radialis

Discussion

To date, research on pain thresholds has been equivocal, with several studies indicating that the pain threshold either rises, declines, or remains unchanged with the aging process. Several investigations undertaken among diverse populations recognized a trend of elevated pain thresholds linked to aging, with older people found to have reduced pain sensitivity irrespective of sex (Bek et al. 2002; González-Roldán et al. 2020). The age-related neurobiological changes result in diminishing functional capability, which in turn affects pain sensitivity as well (Yezierski 2012). The aging process is related to a decline in functionality, structures, nerve conduction velocity, and density of both myelinated and unmyelinated nerve fibers, which are primarily responsible for carrying pain signals (Yezierski 2012). In addition, degeneration of the dorsal horn in the spinal cord and alterations in certain regions of the brain that are particularly specialized in nociception processing, including the prefrontal cortex, primary and secondary somatosensory cortex, anterior cingulate, hippocampus, thalamus, and insula, are associated with aging (El Tumi and Tashani 2017). Also, there is evidence regarding the decreased amount of substance P in elderly people (Gibson and Farrell 2004). All of these age-associated physiological and structural alterations are hallmarks of reduced pain sensitivity in older individuals (El Tumi and Tashani 2017). Contrary to the above-described findings, the present study found lower pain threshold levels in older participants, indicating heightened pain sensitivity among them. However, studies conducted by Pickering et al. (2002), Lautenbacher et al. (2005), and Petrini et al. (2015) validated this contradictory finding. Considerable arguments have been proposed regarding these inconsistent results, among which the utilization of different modalities to evoke pain in human subjects is notable. Generally, studies using heat pain stimulus yielded an age-associated increase in pain threshold (Edwards and Fillingim 2001; Helme et al. 2004), whereas the use of pressure pain stimulus resulted in a trend of decreasing pain threshold along with increasing age in multiple studies (Pickering et al. 2002; Lautenbacher et al. 2005; Cole et al. 2010). According to Lautenbacher and colleagues (2005), the nociception processing in the spinal cord is not similar for pressure and heat pain stimulation. Both deep tissues and superficial tissues are involved when pressure pain is applied, while in heat pain stimulation, superficial tissues are predominantly activated. The descending inhibitory control appears to exert greater influence on spinal input from deep tissue nociception when compared to superficial tissues. As a result of this, the age-associated reduction in endogenous pain inhibition may act more effectively on pressure pain nociception, leading to a decline in age-related endogenous pain inhibition for pressure pain. This ultimately may cause a lower threshold level as well as enhanced pain sensitivity in the elderly (Edwards et al. 2003; Lautenbacher et al. 2005; Cole et al. 2010). In contrast to previous research findings, this study showed that, regardless of sex, middle-aged adults exhibited slightly elevated pain threshold levels compared to younger adults, although the age-related differences in pain threshold were non-significant. However, these levels declined in older age groups, aligning with established trends. A recent meta-analysis concluded that, compared to older adults, younger adults exhibited better pain inhibition, and a decline in pain inhibitory capacity may begin in middle age (Hackett et al. 2020). The different results found in our study may be due to the classification of age groups, since the age categories used in studies are varied. Moreover, the use of different pain modalities and locations to induce pain is not similar.

This study showed that older participants demonstrated a lower mean tolerance value when compared to younger age groups, signifying a decrease in pain tolerance levels with advancing age. The results of other studies correspond with this pattern (Bek et al. 2002; Petrini et al. 2015). It is noteworthy that age-related alterations in pain threshold and pain tolerance manifest bi-directionally, with pain threshold levels tending to increase and pain tolerance levels showing a tendency to decrease with age (Lautenbacher et al. 2017). However, the mechanisms underpinning age-related changes in pain tolerance remain relatively underexplored. One possible explanation could be the diminished capacity to activate the endogenous pain modulatory system in older individuals (Bodnar et al. 1988; Bek et al. 2002). Nonetheless, certain theoretical frameworks regarding pain tolerance posit that pain tolerance levels are significantly influenced by cumulative experience; as individuals age, their exposure to and experience of pain may contribute to enhanced pain tolerance (Anderson and Losin 2016). However, the findings of our current study among the tribal population contradict these studies, as pain tolerance appears to decrease with advancing age in this specific population. This disparity denotes that the pain tolerance levels in this particular population may be impacted more by biology than culture.

This study revealed a significant sex disparity in both pain threshold and pain tolerance levels, with females displaying greater sensitivity to pain compared to males. This finding is aligned with a number of previous investigations (Bek et al. 2002; Pelfort et al. 2015; Lue et al. 2018). Numerous biological mechanisms have been suggested to justify the increased sensitivity observed in females, including the influence of sex hormones and endogenous pain modulation. Enhanced levels of the female gonadal hormone progesterone are linked with decreased pain threshold, whereas estrogen levels are linked to nociceptive modulation (Bartley and Fillingim, 2013; Archey et al. 2019). On the other hand, the testosterone hormone's role in decrementing pain sensitivity has been reported previously (Bartley et al. 2015). A less effective endogenous pain inhibitory capacity in females is one mechanism that has been suggested as an explanation for why females are less sensitive to pain than males (Bulls et al. 2015). Besides these, social factors, such as gender roles and psychological-cognitive variables also contribute largely to this disparity (Belfer 2013). It is essential to recognize that social models and early learning experiences influenced by family and culture play a distinct role in shaping gender-related behaviors (Koutantji et al. 1998). For instance, societal expectations often encourage women to be more aware of pain and express it openly, potentially leading to a greater willingness among women to report pain and, as a result, lowering their pain threshold. Conversely, men are often encouraged to adopt a stoic demeanor, suppressing their pain expression, potentially leading to an increase in their pain threshold (Defrin et al. 2009). It seems that this tribal community also upholds this gender-stereotype perspective towards pain.

In the present study, the interaction effects of age and sex on the pain threshold were non-significant. The insignificant interaction effect of age and sex implies that the trend of an age-associated decrease in pain threshold level is similar in both males and females, even when considering the notable hormonal changes that occur in females during different reproductive events and as they age. While some prior studies (Pickering et al. 2002; Petrini et al. 2015) contradicted this observation by suggesting that age-related changes in pain threshold levels were more pronounced in males than females, a study by Lautenbacher and colleagues (2005) supports this result. Additionally, Lautenbacher et al. (2005) noted that the small sample size of their study might have influenced their findings. However, this proposition doesn't seem significant in light of the large sample size used in the current research. Furthermore, while hormones like estrogen influence pain sensitivity, their impact might be complex and vary across individuals. Other factors might play a more dominant role in age-related pain changes. The similarity in age-related changes in pain threshold levels between males and females can be attributed to shared neurobiological mechanisms governing pain perception. The aging process appears to impact these common mechanisms similarly across genders, resulting in a parallel decline in pain threshold values. This underscores the overriding influence of age-related neurobiological changes on pain sensitivity, transcending sex-specific differences. Further research into the specific neurobiological pathways affected by aging could enhance our understanding of this convergence.

The male participants of this present study exhibited a sharp declining trend in pain tolerance with increasing age, while the females also indicated a similar trend, but no marked difference across the age groups of the females was observed. This finding aligns with the studies by Woodrow et al. (1972) and McEntarfer et al. (2005), indicating that the decline in pain tolerance with age was evident in males but not in females. This finding may be attributed to not considering the menstrual cycle phase in the study for female participants. It is likely that some young and middle-aged females were assessed during lower estrogen phases, possibly increasing pain sensitivity. Inclusion of these women may have lowered average pain tolerance in their respective groups, narrowing the difference in pain tolerance across the three age groups (McEntarfer et al. 2005).

The present study has several limitations. The cross-sectional design employed in the study lacks the capacity to capture individual changes in pain threshold and tolerance with age. Restricting the assessment of pain to a single method (pressure pain) introduces challenges in drawing robust inferences from the study. Furthermore, the observed alterations in pain threshold and tolerance levels are confined to specific muscle points, impeding generalization to other anatomical regions. The current study did not control for the menstrual cycle phases of the female participants. Self-reported pain unpleasantness was not assessed, which could give a better insight. Additionally, variables, such as gender role expectations of pain, the role of family, and participants' upbringing, which could potentially influence pain perception, were not accounted for.

Conclusion

Our research on pain perception among the Santal people revealed substantial effects of age and sex on pain threshold and tolerance levels. Specifically, males exhibited significantly higher pain threshold and tolerance values compared to females. Furthermore, a notable age-related decline in pain threshold and tolerance levels was observed. The interaction effect of age and sex was insignificant for pain threshold levels; however, the interaction effect of age and sex was significant for pain tolerance levels, indicating that the decline in pain tolerance with age was marked in males but not in females. This research primarily underscores the influence of biological factors and, to some extent, socio-cultural factors on pain perception within the Santal tribal community. These findings highlight the importance of considering both age and sex factors when assessing pain perception among the Santal population. In a clinical context, these insights can guide healthcare professionals in tailoring pain management strategies that consider sex and age-specific variations, enhancing the effectiveness of interventions, and contributing to more personalized and culturally sensitive healthcare practices.

Ethics approval and consent to participate

The study is ethically approved by the Review Committee for Protection of Research Risks to Humans, Indian Statistical Institute (ISI Ethics Clearance No.: ISI-IEC/2022/02/03). Written informed consent was obtained from all individual participants included in the study.

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Conflict of interests

There is no competing interest to declare.

Authors' contribution

Both AS and SKR conceptualized the study. Data collection, analysis and interpretation were done by AS. Both SKR and MG reviewed the first draft of the manuscript. The final draft was reviewed by AS, SKR, MG and DC.

Authors' statement

The article has not been previously published or concurrently submitted to an editorial office of another journal, and it has also been approved by all authors and the institutions where it was developed.

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The Facial approximation of the controversial skull attributed to Wolfgang Amadeus Mozart (1756–1791)

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Abstract: Wolfgang Amadeus Mozart (1756–1791) is considered as one of the greatest composers of the Classical Period of music (ca. 1750–1820). Gifted with an unparalleled precocity, which allowed him to play and compose at the highest levels from a very young age, he continued his studies until the end of his life. Despite his prominent status, he was buried in a collective grave and years later his skull was supposedly recovered, reaching the present day surrounded by an atmosphere of mystery and controversy. This study, using a free, open-source, multiplatform software and the available published material, independently seeks to approximate the face of this skull and compare it with previous publications and portraits painted during the composer's lifetime.

Key words: anatomy, anthropology, artificial intelligence, facial approximation, 3D reconstruction, Mozart

Original article

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Introduction: a brief biographical sketch of Mozart

Wolfgang Amadeus Mozart was born in Salzburg (Austria), on January 27, 1756. His father Leopold (1719–1787), who was a violinist, encouraged his sons to pursue music from an early age. Mozart started playing his first chords on the harpsichord at the age of three, performing short pieces at the age of four, and writing his first compositions at the age of five, demonstrating his great precocity. Leopold saw his son's skills as an opportunity for professional recognition and financial gain and in mid–1763 he set off on a tour with his family, performing alongside Mozart and his sister Anna in several European cities, including Munich, Brussels, Paris, and London. In 1769, the Mozart family, this time only father and son, set off on another tour to Northern Italy, which was a very positive opportunity for the young composer, since mastering Italian opera was essential to his career. Subsequently, Mozart returned to Northern Italy on two other occasions, in 1771 and 1772. Leopold had hoped that his son would secure an appointment in Milan, but his expectations were dashed. He did not give up, though, and sought a position for Mozart at the court of Salzburg. Although Leopold did not secure the position, Viennese music seemed to have had a considerable effect on his son, awakening his creative genius. In 1774 Mozart was appointed *Konzertmeinster* at the court and received a salary for this work. However, the job was not very demanding and did not meet either his abilities or ambition, which encouraged him to seek new opportunities. In 1777, after requesting release from the post, he left with his mother for other cities in order to apply for other positions. They went to Munich and Mannheim, where they did not find much work, but in the meantime Mozart met and fell in love with Aloysa Weber (ca. 1760–1839). However, the young *soprano* did not reciprocate the composer's feelings so the young Mozart, accompanied by his mother, left for Paris, where he quickly found a job. However, his luck also rapidly changed with the death of his mother. Discouraged, he returned to Salzburg in 1780, where he found some success, and later to Munich, establishing himself as a respected composer. Around 1782 Mozart married Constanze Weber (1762–1842, Aloysa's sister), and this period coincided with his estrangement from his father, Leopold. Living in Vienna from 1784 onwards, he enjoyed great prestige and inspiration. Although his income was higher than that of the average musician in his position, his financial extravagance forced him to find ways to control his spending. It was during this period of financial instability that he composed *The Marriage of Figaro* (1786) and *Don Giovanni* (1787), his most famous operas. Leopold died in May 1787. This period coincided with the great success Wolfgang enjoyed with his performances in Prague, where his premieres attracted large audiences and were received with great enthusiasm. In 1791, after some tribulations, things seemed to have improved for Mozart. It was in that year that he presented the opera *The Magic Flute*, which was a great success and would become the most beloved on the stage. In 1791 he also began writing his *Requiem*, which he left unfinished as he died on December 5, 1791 almost at the age of 36 from "severe miliary fever". He was buried in a multiple grave and a small group of friends were present at the funeral. Constanze, from whom he had six children, remarried and, together with her second husband, she worked to keep Mozart's memory alive. She died in 1842 at the age of 80 and had the opportunity to witness the recognition of the late great composer's work (Sadie 2024).

The Skull Attributed to Mozart

According to one version of the skull's story, which is generally the most widely accepted, there were two gravediggers present at Mozart's burial, and one of them, Joseph Rothmayer, marked the location of the musician's coffin (Murray 1993). Years later, when the space was cleared to accommodate new residents, the gravedigger, knowing the location of the skull, recovered it and kept it (Karhausen 2001; Eng 1906). Years later, he handed the skull over to his successor, Joseph Radschorpf, who in turn gave the piece over to the musician Jacob Hyrtl (1799–1868). When Hyrtl died, the skull was acquired by his brother, the anatomist Josef (1810–1894) (Murray 1993). Between 1895 and 1900, it is not known what happened to the skull, and in 1901 Joseph Schöffel (1832–1910), the curator of the *Hyrtl Foundation*, declared that the anatomical piece that had mysteriously disappeared had been found in one of the foundation's buildings. The skull was then officially donated to the *Mozarteum* in Salzburg on March 11, 1902 (Karhausen 2001).

There is some controversy surrounding the authenticity of Mozart's skull, including the difference in the tooth count. For example, the *Mozarteum* piece has 11 teeth and the description made by the writer and poet Ludwig August Frankl (1810–1894), a friend of Joseph Hyrtl and a witness, counts only 7 dental elements in the jaw. However, Eng's work (1906) describes Frankl's work as "superficial and fleeting" and endowed with "imaginative exaggerations" due to "excitement" and in the end, indicates that the skull would be authentic (Eng 1906).

Later works attempted to prove or refute the authenticity of the skull (vd. Tab. 1). In 1957, the embryologist Gustav Sauser (1899–1968) expressed the view that the skull was not Mozart's, while in the same year, the anthropologist Ämilian Kloiber (1910–1989) gave a positive opinion on its authenticity. In 1963, Carl Bär argued negatively about its attribution (Karhausen 2001).

Table 1. Studies on the skull's authenticity

Author	Approach	Yes	No	Inc.
Eng and Minnich (1906)	Macroscopic examination	X		
Sauser (1957)	Macroscopic examination		X	
Kloiber (1957)	Macroscopic examination	X		
Bar (1963)	Macroscopic examination		X	
Puech et al. (1987)	Macroscopic examination	X		
Kritscher et al. (1989)	Macroscopic examination	X		
Murray (1993)	Literature review		X	
Karhausen (2001)	Literature review		X	
Parson (2006)	Genetic analysis			X

Between the late 1980s and early 1990s, the anthropologist François-Pierre Puech and his team developed a series of studies that sought not only to assess the authenticity of the skull, but also to address other anthropological aspects. According to one of the studies, the skull was Mozart's, and it even structurally matched a portrait of the composer made in 1778 (Puech et al. 1989). Based on the team's assessment, the skull would have belonged to a male individual, albeit gracile, between 25 and 40 years of age. Mozart would also have been ca. 1.50–1.52 m tall with a brain capacity of 1585 cm^3 (Puech et al. 1989b). They discovered what was interpreted as a calcified extradural hematoma on the left temporo-parietal endocranial surface (Puech et al. 1989c). A forensic facial approximation was performed, indicating a high compatibility with known portraits of the composer (Puech 1991).

In another study, Kritscher et al. (1989) analyzed the skull at the request of the *Mozarteum* and determined that it belonged to a male, aged between 25 and 40 years. The researchers also made a forensic facial approximation using the Russian method of Mikhail Mikhaylovich Gerasimov (1907–1970) and, when comparing the face with the portrait, the structural similarity was quite significant. The final indication was that the skull was probably authentic (Kritscher et al. 1989). Two works based on third-party publications substantiated the analysis of Murray (1993) and Karhausen (2001) with both indicating the inauthenticity of the skull.

In some of the previous publications, the authors indicated that there was a need for DNA testing to increase the level of accuracy of the findings, but none had worked with such an approach until, finally, in 2006 a documentary produced by the Austrian television station *ORF* presented the results of such an examination. Researchers at the Innsbruck Institute of Medicine collected biological material from skeletons attributed to Mozart's niece, Janette, and maternal grandmother, Euphrosina. They also extracted material from two teeth from the skull attributed to Mozart, but the final result was inconclusive, as none of the samples were related to each other (Black 2012; Harding 2006).

This our study is independent and has no connection with the institution that preserves the remains of Wolfgang Amadeus Mozart, nor with the universities and institutions that previously performed the examination on them. The motivating element of the article is the creation of didactic material to explain the facial approximation technique, by testing the possibility of reconstructing a face using data originally available in newspaper articles, online media, books and academic journals. In addition, it offers a comparative analysis that may help elucidate the mystery surrounding the attribution of this skull.

Materials and methods

Concepts, Software and Hardware

Forensic facial reconstruction (FFR), also known as forensic facial approximation (FFA), represents an auxiliary recognition technique addressing the approximation of individuals' facial morphology beginning from their skull. It is used when not sufficient information is available for personal identification (Stephan 2015; Pereira et al. 2017). It should be highlighted that this set of methodologies does not mean identification per se, as it would be possible through DNA testing or through a comparative examination of dental arches, yet it deals with the recognition by people observing the produced image that may subsequently lead to identification (Baldasso et al. 2020).

This work implements the step-by--step approach discussed by Abdullah et al. (2022) and Moraes and Beaini (2024) and Moraes et al. (2024). This technique starts with the configuration of the skull in the 3D scene, followed by the projection of the profile and facial structures on statistical data, hence generating the volume of the face with the aid of the anatomical "deformation" technique (Quatrehomme et al. 1997) and concludes with producing the facial details, with a full configuration of the hair, clothing and the ultimate generation of the definitive images.

The modeling process was performed in the *Blender 3D* software, running the *OrtogOnBlender* add-on (website: http://www.ciceromoraes.com.br/doc/pt_br/OrtogOnBlender/index.html) and its submodule *ForensicOnBlender* (Pinto et al. 2020), both developed by the first author of the article. The program and the add-on are free, open source and multiplatform, and can run on Windows (>=10), MacOS (>=BigSur) and Linux (=Ubuntu 20.04).

A desktop computer with the following characteristics was used: Intel Core I9 9900K 3.6 GHZ/16M processor; 64 GB of RAM; GeForce 8 GB GDDR6 256-bit RTX 2070 GPU; Gigabyte 1151 Z390 motherboard; SSD SATA III 960 GB 2.5"; SSD SATA III 480 GB 2.5"; Water Cooler Masterliquid 240V; Linux 3DCS (https://github.com/cogitas3d/Linux3DCS), based on Ubuntu 20.04.

Forensic Facial Approximation

To perform a FFA, it is essential to possess a series of data about the skull. This includes photographs in different views, measurements and anthropological analyses. In some cases, the availability of radiographic images, imaging tests and other data.

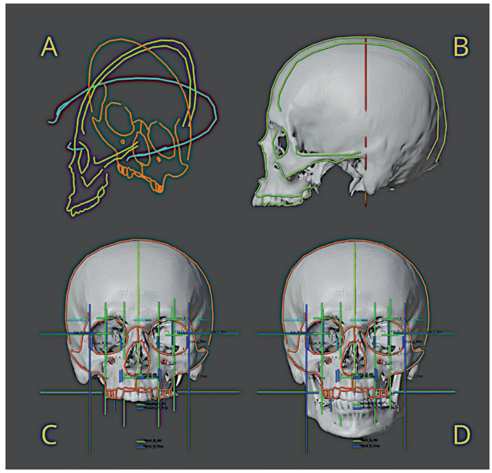

Fig. 1. A-D: Skull reconstruction

In this study, information available in the publications of Puech et al. (1987), Puech et al. (1989) and Kritscher et al. (1989) was used, which allowed two-dimensional projections of the Mozart's skull. It was possible to make projections in the front (X and Z axes), lateral (Y and Z) and superior or top (X and Y axes) views (Fig. 1A). Such projections are made by drawing the outline of the images, giving an adequate scale, adjusted with reference to the measurements described. The skull of a virtual donor was imported and adjusted to fit the limits informed by the consulted references (Fig. 1B). Since the mandible was missing, it was necessary to position some anatomical points and project the measurements of structures related to the soft tissue and the skull itself, among them the inferior limit of the mental protuberance. These projections are based on measurements taken from computed tomography scans of living individuals and different ancestries (Moraes et al. 2021; Moraes and Suharschi 2022). Again, in Mozart's case, it can be seen that the projection from the frontomalar orbital distance generates a mental protuberance lower (on the Z axis) than the average for adults, denoting that the skull is proportionally larger on the X axis than on the Z axis (Fig. 1C). With the projections of the lower limit of the mental protuberance available, the virtual donor's mandible was adjusted to fit the pattern presented at the lower limit of the incisors, which in Mozart was smaller than the average and the distance in relation to the mental protuberance remained compatible (Fig. 1D). It is important to highlight that, in addition to the projection of the mandible limits, the skull provided information on fitting (mandibular fossae) and occlusion (maxillary teeth). These structures allow the reconstructed mandible to have more structural coherence, being complemented by information extracted from measurements of cranial samples. Therefore, these are not random choices, but based on anatomy and statistics: more details will be covered in the *Results and Discussion* section.

Two video lessons on the projection methodology are available online at: *lesson 1* (https://youtu.be/U6oYkEmfyWo), *lesson 2* (https://youtu.be/Vcz2e5uSFX8).

Soft tissue thickness markers were distributed over the surface of the skull (Fig. 2A), following the table of measurements related to European males with average BMI (De Greef et al. 2006). Nasal projection was performed using three different data, the projection by the Russian method, the Manchester method and the complementary methodology developed by the authors of the present work together with a team of experts. A video lesson on the approach can be accessed online (https://youtu.be/F205kLQ--Oo). With the data on soft tissue thickness and nasal projection, it was possible to trace the profile of the face (Fig. 2B).

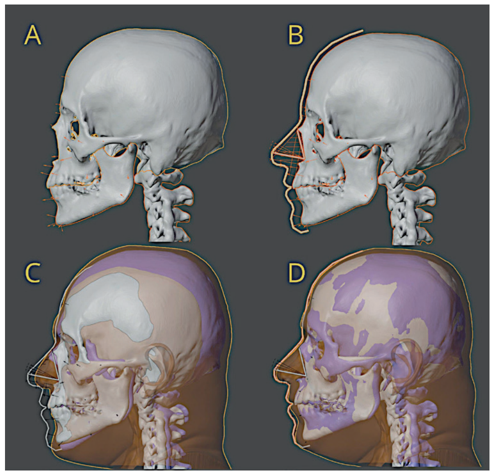

Fig. 2. A-D: Initial steps of the facial approximation

To complement the structural data, the tomography of a virtual donor, reconstructed in *OrtogOnBlender* itself (Moraes et al. 2021c), was positioned in the same plane as Mozart's (Fig. 2C) and adjusted so that the donor's skull matched the one that would be approximated (Fig. 2D), reflecting the deformation in the soft tissue and, therefore, generating a face structurally close to what it would be in life (Quatrehomme et al. 1997). In the process, it was possible to segment the structure corresponding to the endocranium. A video lesson addressing the anatomical "deformation" can be accessed online (https://youtu.be/xig5_EcIFWA).

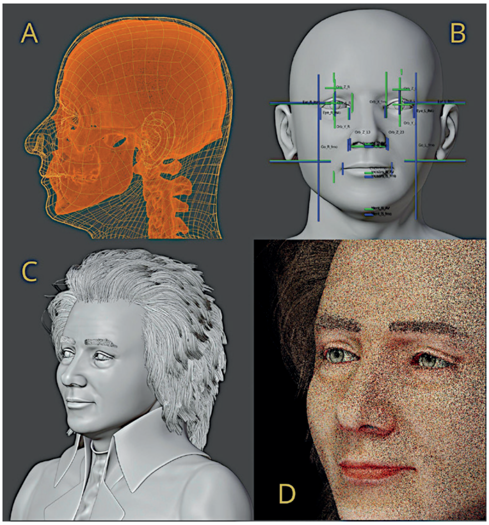

Fig. 3. A-D: Final steps of the facial approximation

Following the approach available in Abdullah et al. (2022), a previously prepared bust was imported and distorted based on the interpolated data from the projections and anatomical deformation (Fig. 3A, B). The expression marks were then digitally sculpted to match the face with the composer's age at the time of his death; the clothing, wig and other facial hair were also modelled (Fig. 3C). For the lighting and pigmentation of the skin, a series of images related to Mozart available on the *Wikimedia Commons* website were taken as reference (Fig. 3D). After the face was completed, comparisons and measurements were made and images of the face were generated.

The final facial images were refined using artificial intelligence (AI) to sharpen facial details such as expression lines, correct eyebrows and skin tone (Fig. 4). All processing was performed offline using the Stable Diffusion web UI tool (https://github.com/AUTOMATIC1111/stable-diffusion-webui) and manual image editing with the *Gimp* software (https://www.gimp.org) was performed to correct some inconsistencies. Care was taken to ensure that the improved regions maintained a structure compatible with the original image.

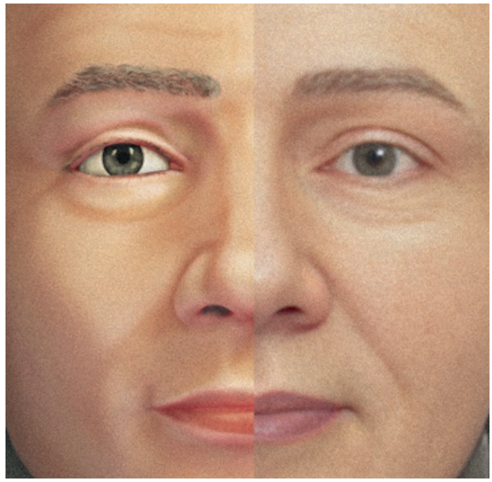

Fig. 4. Original on the left and AI+manual edit on the right

Results and Discussion

It is possible to compare the projections made in the present work (Moraes et al. 2024) and that of (Kritscher et al. 1989) and both are smaller than the average for adults and even smaller compared to the proportion from the fmo-fmo distance (Fig. 5). There is no description of which method was used for the projection of the mandible in the publication by Kritscher et al. (1989), making it difficult to understand the approach chosen by the authors. However, this projection is made in relation to the drawing presented and not to the sculpture, which apparently used the mandible of a donor and may have differed from the drawing in dimension, as will be seen below.

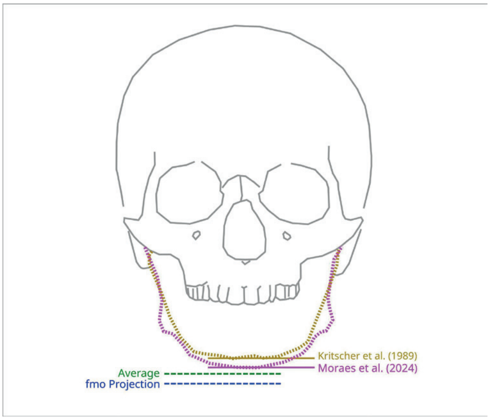

Fig. 5. Comparison of mandibular projections

Regarding the functional issue of mandibular projection and its limitations and effective use in facial approximations, it is possible to find some approaches in the forensic literature that illustrate this question. Taylor (2000) uses the Sassouni and Krogman projection: although the author admits that this technique is not 100% accurate and that it is based on a normal skull, without potential structural deformations, she presents successful cases of facial approximation that led to subsequent identification, demonstrating that even in the forensic context, the absence of a mandible is not an impediment to the facial approximation procedure. In another work, Wilkinson (2004) points out potential problems in the projection of the mandible in relation to other missing regions; however, the study she cites has only 6 structural reconstructions based on just one skull, unlike the projections used in the present work, which are raised from samples ranging from 75 to 110 skulls (Moraes and Suharschi 2022). The technique was tested during the approximation of the face of Zlatý kůň, a fragmented skull that was reconstructed from another approach and whose results were quite similar, indicating that the projection of the mandible used in this work, in addition to anatomical and statistical coherence, also presents results that converge with other reconstructive approaches (Moraes et al. 2024). Although the ideal scenario for facial approximation involves an entire skull, in cases of structural absence, such as the one presented here, reconstruction techniques are not only applicable, but also useful in situations of great gravity and seriousness, such as those related to the approximation of crime victims, in an effectively forensic context.

Fig. 6. Upper images: Comparison between the portrait painted by Joseph Lange and the facial approximation in this work. Picture credit: *Wikimedia Commons - Mozart-Lange.jpg*. Lower images: Comparison between the portrait drawn by Dora Stock and the facial approximation in this chapter. Picture credit: *Wikimedia Commons - Mozart drawing Doris Stock 1789.jpg*

In relation to Lange's work (1782–1783), a mask of the approximation was positioned on the face and was significantly compatible, differing slightly in the region of the forehead and chin, where the structure of the approximation was shown to be more projected than that of the painting. However, the projections of the nose, eyes and the position of the lips are quite similar in both approaches (Fig. 6, upper image).

When the approximation is compared with Stock's profile drawing (1798), the compatibility is significantly greater in the region of the nose, lips, eyes, forehead and even the ear (Fig. 6, lower images). The region with the greatest incompatibility is the mental one, but it should be remembered that this is a general mandibular projection, respecting what is expected of a multi-ancestral population.

Fig. 7. Comparison between the portrait and the facial approximations

Since Stock's drawing (1789) was made in profile, it ended up allowing the comparison of all facial approximations made to date, since all of them have captures from the same point of view. The work of Kritscher et al. (1989) is generally compatible with the entire face, except for the chin region, where it projects a little more, perhaps because it is the version related to the physical sculpture, with the jaw of a donor, and not to the two-dimensional drawing. The work of Puech (1991) is also generally compatible, with a small difference in the tip of the nose and the upper lip, both a little more projected. The current work is generally compatible with the face, although it differs in the mental region, which is more projected. Although all the approximations have small differences in different parts, they all indicate a pattern quite similar to the face portrayed by Stock in 1789 (Fig. 7).

However, this could not serve as a proof that the skull definitely belonged to Mozart. Since the forensic facial approximation technique aids in recognition, not strictly in identification, so it may happen that similar skulls result in facial approximations that resemble the faces of different individuals. Furthermore, there is no denying that there is a great compatibility and, since the aDNA test did not answer the questions related to identification, it remains to speculate that it could be the composer's skull, or that it could be a great coincidence, arising from a potential structural compatibility of the region's population at that time as discussed in Karhausen (2001).

Table 2. Endocranial volume

Author	Volume (cm^3)
Puech et al. (1989)	1585
Kritscher et al. (1989)	1388
Moraes et al. (present study)	1447

Regarding brain capacity, the study by Puech et al. (1989b) estimated it at 1585 cm^3, using the mustard seed filling method, but it was not very clear how this was done, since part of the anatomical structure is missing. The survey by Kritscher et al. (1989) used Lee Pearson's general ancestry formula to calculate what the capacity could have been, resulting in 1388 cm^3. In this study, the approach used was the segmentation of the endocranium based on anatomical deformation, which took into account data on skull thickness in the works consulted. Since the anatomical deformation used a complete skull and this was in accordance with the dimensions of the references, the final volume was 1447 cm^3. When applying the conversion of the endocranium to brain volume, reducing the value by 9.81% (Moraes et al. 2023), the volume is 1305 cm^3, which falls within the standard deviation for modern men, which is 1234 cm^3 (± 98) (Ritchie et al. 2018). As for the head circumference, the measurement resulted in 54.16 cm, closer to the average for women, which is 54.3 cm (± 2.3), compared to men, which is 56.2 cm (± 2.4), although it falls within a standard deviation of the second (da Costa et al. 2021).

Six images were rendered for the presentation of the face:

- Three poses in the objective version, in grayscale, as there is no information on skin color; with eyes closed, as the shape of the open eyes is not known exactly; without hair and facial hair, due to the same lack of information and without clothing and accessories (Fig. 8). These images did not receive final artwork with AI.
- The other three images contain subjective and more artistic elements, such as skin color and eyes, which are open; hair, accessories and clothing (Figs. 9 and 10). These images received final artwork with AI + manual editing.

Fig. 8. Objective facial approximation rendering

Fig. 9. Complete – Three-quarter view

Fig. 10. Complete – Frontal and profile views

Conclusions

This work was the first proposed case of purely digital and three-dimensional facial approximation of the alleged Mozart skull. It was possible to compare the results with previous approximations, which showed convergence, serving as an illustrative case of structural coherence in forensic facial approximation, regrardless of applying approaches based on different techniques introduced in different decades. The use of artificial intelligence, performed with human monitoring and manual adjustments, allowed a significant increase in details, without clashing with the raw renderings, demonstrating that the use of these tools, instead of distorting the work of an expert, can be an important aid in improving graphic quality. Because it is an approach focused on the use of open source software, aiming at educational purposes for the field of forensic facial reconstruction, this work also served as a source of data for the replication of techniques, by sharing not only the step-by-step process, but also teaching tools that, together, allow the replication of the process by potentially interested parties.

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Conflict of interests

The authors have no conflict of interests.

Authors' contributions

CM: conceptualization, writing-first draft and revision; JŠ, ME.H, LS, TB: critical review of the first draft, writing-revision, literature search, methodology; EV, FMG: writing-first draft and revision, supervision.

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Variability of anti-Müllerian hormone and folliculotropic hormone levels in women of reproductive age in relation to normal or impaired ovarian function

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ABSTRACT: The demographic crisis in Europe is growing due to an increasing proportion of couples with fertility disorders. The purpose of this study was to examine the variability of ovarian reserve markers with age in women with premature ovarian insufficiency (POI) and polycystic ovary syndrome (PCOS) in relative to women with normal ovarian function. Two hormones were analyzed: anti-Müllerian hormone (AMH) and folliculotropic hormone (FSH). This study demonstrates that AMH is a valuable indicator of alterations in reproductive capacity. FSH is a standard marker of the hypothalamic-pituitary-ovarian axis.

We examined the reproductive status of 390 women aged 23–46 in three groups. Ovarian dysfunction was determined by medical diagnosis. The study includes women with PCOS (n=154), POI (n=40), and control group (n=196) with normal ovarian function (NOF). Blood samples were collected to measure AMH and FSH. We used multivariate logistic regression analysis to demonstrate the relationship between hormone levels and age in different age groups. ANOVA was used to analyze factors related to AMH and FSH concentrations. The results confirmed that women with POI had significantly lower AMH concentrations and higher FSH concentrations than women with normal ovarian function only in the group of women aged 36–46 years. There were no statistically significant differences in FSH levels in women with POI and NOF in the 23–30 and 31–35 age groups. AMH levels were higher in the PCOS group than in women with NOF in all age groups. FSH marker did not differ compared to the control group in women aged 23–30 and 36–46. The predictive value of AMH in the diagnosis of PCOS is significantly higher than the commonly used FSH. The results may contribute to earlier assessment of biological status to support reproductive chances in women with POI and PCOS.

KEY WORDS: AMH, FSH, reproduction, fertility, PCOS, POI

Original article

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Introduction

European reports indicate an increasing prevalence of infertility among couples of reproductive age (EPAF – European Policy Audit on Fertility 2024). The European Society of Human Reproduction and Embryology recommends setting up research projects to improve reproductive health. In this study, we investigated the variation of anti-Müllerian hormone (AMH) and folliculotropic hormone (FSH) in relation to age among women with normal and impaired ovarian function such as premature ovarian insufficiency (POI) and polycystic ovary syndrome (PCOS). Endocrine regulation of the sexual cycle determines the normal functioning of the reproductive system. The AMH level is a conservative indicator of reproductive potential and is therefore one of the most stable markers of ovarian reserve. AMH is an indicator for early diagnosis of ovarian dysfunctions such as POI and PCOS. In the current literature, there are still too few data related to AMH and FSH variability in women with POI and PCOS. In this study, we investigated the variability of hormones in women with ovarian dysfunction in relation to chronological age.

The importance of AMH and FSH in the development of ovarian reserve

Differentiation of the reproductive system during organogenesis follows two different pathways depending on sex determination. The initiation of AMH transcription is strictly controlled in terms of location and timing. During embryogenesis, transcription factors regulate sex-determined pathways for the hermaphrodite gonads (Svingen and Koopman 2013). During the sixth week of organogenesis, Wolff ducts differentiate. Under the influence of maternal estrogen, paired Müller ducts are formed. In the 10th week, in the absence of the Y chromosome, hermaphrodite gonads have not initiated the male pathway program in the second trimester of pregnancy. Consequently, they begin differentiation into ovaries by activating the expression of Wnt4 and Rspo1 genes.

The Wolf ducts degenerate, and the Müller ducts differentiate into fallopian tubes, the uterus and the upper vagina. Activation of the secretion of gonadoliberin in the hypothalamus stimulates the activity of the pituitary gland which, via the gonadotropic hormones FSH and LH, initiates the formation of ovarian reserve. The presence of AMH is detectable at 24 weeks of gestation in the granulosa cells of preantral follicles (Kuiri-Hanninen et al. 2011). The pool of ovarian follicles formed during fetal development will constitute the ovarian reserve of a woman of reproductive age (Nelson et al. 2007; Kwee et al. 2008; Blińska and Hejmej 2013).

Structure and function of AMH and FSH

The reproductive period of women is determined by ovarian reserve. The hormones AMH and FSH regulate the recruitment of ovarian follicles during folliculogenesis (La Marca et al. 2009; McGee and Hsueh 2006). AMH is responsible for managing the pool of follicles during their recruitment to the preantral follicle stage. Further follicle maturation occurs under the control of FSH. During ovulation, an oocyte is released from the Graaf follicle, and the follicular phase transitions to the luteal phase of the menstrual cycle.

AMH is a dimeric glycoprotein composed of 560 amino acids. Due to the presence of a C-terminal domain, it belongs to the β-TGF superfamily of transforming growth factors (Cohen-Haguenauer et al. 1987; Knight and Glister 2006; La Marca et al. 2009). The gene encoding AMH is located on chromosome 19p13.3.

Activation of the precursor form of AMH determines binding to the AMHR-II receptor and activation of the signaling cascade in target cells through tyrosine phosphorylation and activation of SMAD signaling proteins (di Clemente et al. 2010).

The number of AMHR receptors and their location are variable and dependent on age. The AMH-receptor system and the control of the menstrual cycle by the hypothalamic-pituitary-ovarian (HPO) axis determine reproductive fitness from menarche to the depletion of ovarian reserve. With the loss of follicles, AMH levels decline and expire (Kerkhof et al. 2010; Hagen et al. 2010; Kelsey et al. 2011; Lee et al. 2012; Lie Fong et al. 2012).

FSH is produced by basophilic cells of the anterior pituitary lobe under the influence of increased gonadoliberin (GnRH) release in the hypothalamus. FSH in the presence of estrogen regulates the formation of receptors for FSH (FSH-R) (Macklon and Fauster 2011). FSH affects the granulosa layer, resulting in the growth and development of the ovarian follicle (Skałba 2008; Gardner and Shoback 2011). FSH also stimulates an increase in aromatase activity, which is essential in the conversion of androgens to estrogens (Grossman et al. 2008).

FSH with androgens and non-ovarian factors, controls the qualitative and quantitative recruitment and selection of antral follicles to the dominant follicle stage before ovulation. A reduction or absence of FSH results in a decrease in the number of maturing follicles (Dewailly et al. 2016, Kumar et al. 2018).

FSH is a glycoprotein consisting of two peptide chains α and ß. The α subunit (89 amino acids) is characteristic of other gonadotropins. The ß subunit (115 amino acids) defines the specificity of each of the gonadotropic hormones (Gardner and Shoback 2012). The gene encoding FSHß is located on chromosome 11p13. and can be subject to mutations that affect the process of steroidogenesis or follicle recruitment in the ovary.

AMH levels reflect the secretion of only those follicles that are vascularised. AMH has auto- and paracrine effects. Follicles with impaired vascularisation are subject to atresia. At 18 weeks gestation, there are approximately 7 million follicles in the fetal ovaries (de Velde and Pearson 2002). As a result of atresia, the pool of ovarian follicles is reduced to approximately 1–2 million at birth.

At the time of menarche, there are 400,000 follicles in the ovaries and only 1,000 at menopause. The age of onset of natural menopause varies by population and in Poland falls, on average, at 51.2 years of age (Kaczmarek 2007).

POI

As the ratio of primary and primordial follicles to the rarer pre-antral and antral follicles change with age, AMH levels decline and a high percentage of follicles undergo degenerative processes. This results in a decrease in ovarian volume and physiological (menopause) or accelerated cessation of ovarian activity – POI (Urutia et al. 2019). In women with POI, AMH levels decreased with age in all analyzed age groups.

Endocrine and metabolic abnormalities related to the HPO axis may contribute to POI. Elevated FSH levels are a response to poor follicular development during folliculogenesis. Too low estrogen levels affect increased FSH secretion, causing a change in the ratio of LH to FSH, a decrease in the level of sex hormone-binding globulin, and consequently a disruption of ovulation or the occurrence of non-ovulatory cycles.

PCOS

The negative impact of both endogenous and exogenous factors has been identified as a potential cause of PCOS. This endocrinopathy, which is most commonly diagnosed among women of reproductive age, is characterized by high AMH and androgen levels. The excess hormones block the passage of follicles to the next stage of development. There is an accumulation at the preantral and early antral stages (Carlsen et al. 2009; Cessar et al. 2014; Pigny et al. 2003; Piltonen et al. 2005; Eldar-Geva et al. 2005; Homburg et al. 2013; Tal et al. 2014). In patients with PCOS, an increase in granulosa layer mass was observed in the granulosa cells of ovarian follicles. Increased AMH release by granulosa cells affects the cells of the inner follicle sheath, disrupting the conversion of androgens to estrogens. This causes hormonal imbalance and promotes the onset of PCOS symptoms (Ingraham et al. 2000). One of them is the difficulty of achieving pregnancy.

Material and methods

Participants

A total of 390 women were included in the study after giving voluntary and written consent to participate. The study was conducted in the period 15.07.2015 – 31.06.2016. The study group consisted of women aged 18–46 years without general medical treatment for at least one month prior to participation in the present study.

Accepted exclusion criteria are pregnancy and breastfeeding, cancer, endocrine disease, hormone therapy, hormonal contraception, removed uterus or ovary, thyroid disorders, and abnormal prolactin levels.

Based on clinical diagnosis, women were allocated to three groups: PCOS (n=154), POI (n=40), and a group with NOF (n=196). The control group consisted of women who had no abnormalities in reproductive function and came to the clinic because of problems occurring on their partner's side. The group was divided into three age cohorts: 23–30, 31–35, and 36–46 years. Serum AMH and FSH levels were determined using the ECLIA immunoassay method in the laboratories of the Department of Infertility and Reproductive Endocrinology of the GPSK UM in Poznan and the InviMed European Motherhood Centre in Wrocław. Three categories of ovarian function were determined in the study: NOF, POI, and PCOS, and an indicator was adopted – serum AMH levels in the reference range for NOF women: 1.0–3.2 [ng/ml], POI < 1.0 [ng/ml] and PCOS > 3.2 [ng/ml]. The reference values for hormones were established in accordance with the prevailing laboratory guidelines at the time of the study. The study was conducted in a clinical department with the approval of the director of the Department of Gynaecology, Obstetrics, and Gynaecological Oncology, University of Medical Sciences, Poznan and accepted by the local Bioethics Committee.

Collection of material

The object of quantitative analysis is blood serum. For the determination of AMH and FSH profiles, a single blood sample was taken under ambulatory conditions.

Due to the variability in the menstrual cycle, FSH was determined from a blood sample taken in the morning, fasting, on days 2–3 of the menstrual cycle.

Statistical analysis

The data obtained from the surveys were subjected to statistical analysis in the program package STATISTICA 13.3 statistics (StatSoft, Inc., 2014). The number of categories for each factor was determined by the value n, which corresponds to the frequency of hormone determinations of the hormone study. A significance level of $p < 0.05$ was used. Sociodemographic status data and their number and percentage were included in the basic descriptive statistics. Relationships between categorical variables were compared using the $\chi 2$ test. The quantitative variables included in the groups were described by mean ± standard deviation (SD). ANOVA test was used to assess differences between groups. The effect size was evaluated using partial eta squared ($\eta 2$) and classified as: no effect = 0 to 0.039, minimum effect = 0.04 to 0.24, moderate effect = 0.25 to 0.63, and strong effect = $\geqslant$ 0.64. Regression and r-Pearson correlation analysis were used to assess the degree of association between hormone concentrations and modulating factors. The mean age of women with POI= 35.9 (±4.4) years, with PCOS=33.2 (±4.2). Of the 461 women, 71 did not consent to participate in the study or were over the age of 46 years. Mean baseline AMH levels in women with POI and PCOS were 0.56 (±0.3) ng/ml and 6.84 (±6.08) ng/ml, respectively. Mean FSH levels on cycle day three were 3.62 (±11.30) mlU/ml for POI and 6.04 (±1.79) mlU/ml for PCOS. Women were divided into three age cohorts: 23–30, 31–35, and 36–46 years. Most women with POI (60%) were aged 36–46 years with mean values of AMH 0.49 (±0.32) ng/ml, FSH taken on day three of menstrual cycle 10.96 (±14.42) mlU/ml. Most women with PCOS (44.81%) within the 31–35 age range exhibited AMH levels. The study revealed that most women with PCOS (44.81%) within the 31–35 age range exhibited AMH levels of 6.76 ng/ml (±4.04), FSH 10.96 mlU/ml (±1.61). In women with PCOS, AMH levels demonstrated a statistically significant increase with age, reaching a peak in the 36–46 years age group. The mean FSH value (5,94±1,65 – 6,10±1,61) in women with PCOS remained consistent across all age groups, demonstrating no correlation with age.

Results

Characteristics of women with regard to ovarian function status

To trace the changes associated with depletion of ovarian reserve in women with normally functioning ovaries, the study group was divided into three age ranges: 23–30, 30–35, and 36–46 years. The division adopted provided for the comparison of parameters describing the condition of ovaries with POI and PCOS with the NOF group and to assess changes with age. Among women with PCOS, the largest group was between 31 and 35 years (45%). The number of youngest and oldest women was identical (27,5%).

The smallest number of women with NOF was in the 23–30 years group (18.4%). The ranges 31–35 (40.3%) and 36–46 (41.3%) years had similar numbers. Due to the accelerated loss of ovarian follicles in women with POI, the age of menopause will shift and occur earlier than the median age of physiological menopause in Poland of 51.25 years (Kaczmarek 2007).

Variation in AMH and FSH levels among women with POI, PCOS, and NOF

The result of comparing the mean AMH concentration values in the impaired (POI, PCOS) and normal (NOF) groups indicated a significant difference between the groups ($p < 0.01$). A similar result was obtained when comparing the mean values of FSH concentrations in the study groups ($p<0.01$).

The regression analysis performed to determine the age-dependent variability of AMH and FSH concentrations revealed the differences between mean hormone concentrations in subjects with POI and PCOS compared to the NOF group in all age categories (Fig. 1). Results showed a negative correlation between age and AMH levels in the NOF group ($r = -0.31$, $p < 0.05$). A similar results was observed in women with POI, where the decline in hormone levels with age was even more evident ($r = -0.40$, $p < 0.05$). In the PCOS group, there was no significant association between mean AMH levels and age ($r = -0.01$; $p>0.05$), where the concentration of the hormone under study remained stable.

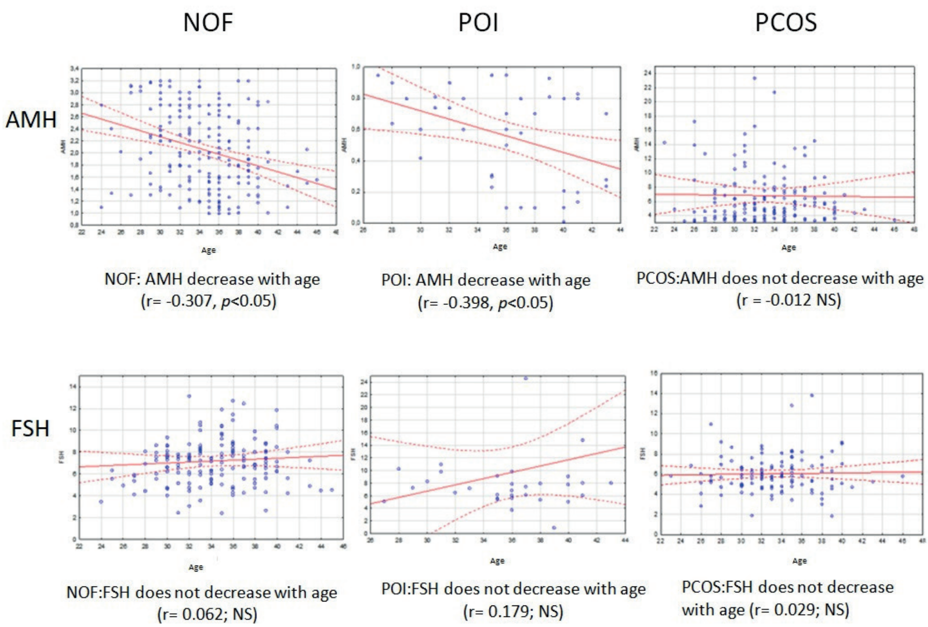

Figure 1. Variability of AMH and FSH levels among women with NOF, POI and PCOS

*NOF-normal ovary function; POI-premature ovarian insufficiency; PCOS-policistic ovarian syndrome; NOF: normal ovarian function

Regression analysis indicated a lack of association between FSH concentrations and age in both women in the ovarian dysfunction groups: POI ($r=0.18$; $p>0.05$) and PCOS ($r=0.03$; $p>0.05$), as well as control ($r = 0.06$; $p>0.05$). While not statistically significant, the regression model for the POI group indicates that there is a tendency for FSH levels to increase with age. In women with NOF, there was a statistically significant inverse correlation between age and AMH concentrations ($p<0.01$), which is consistent with the physiological norm. Conversely, the analysis demonstrated that FSH concentrations remained stable across all age ranges ($p>0.05$).

In the POI group, FSH levels also did not change with age ($p>0.05$), while the results showed a negative correlation in relation to AMH levels and age ($p<0.05$). Furthermore, it was observed that the oldest group of women (60%) had critically depleted ovarian reserve compared to the youngest and middle group. A comparison of mean AMH levels showed that in the 23–30 years group, POI women had lower AMH levels than NFO women at the same age (0.72±0.20 vs. 2.42±0.69g/ml).

However, there was no statistical significance ($p>0.05$) in the analysis of mean AMH levels between women in the POI 31–35 years group and the NFO group. Also, the comparison of predictor levels between POI and NFO in the 36–46 age range did not show statistical significance ($p>0.05$), although a clearer downward trend was observed in POI women.

AMH analysis in a group of women with POI in three age ranges: 23–30, 31–35 and 36–46 years showed that serum AMH levels observed in the youngest group of women were approximately threefold lower than those observed in the NOF group.

A comparable outcome was observed in the 31–35 age group, wherein the mean AMH concentration among women with POI was also threefold lower in comparison to the control group. The observation in the oldest group confirmed that the mean hormone level in women with POI aged 36–46 years was already four times lower compared to women with NFO of the same age. The linear decrease in AMH levels in POI women relative to NFO women with age was observed.

A certain limitation of the variable testing was the slightly lower number of POI women compared to the other groups, however, despite not reaching statistical significance ($p>0.05$), the observation of a trend was as expected.

The observation of AMH variability with age is confirmed by the negative correlation with FSH in the age ranges analyzed.

The analysis of mean FSH levels in women between the ages of 36 and 46 with POI confirms the elevated mean concentration in the NOF group. The mean FSH level is 1.5 times higher than that observed in the control group ($p<0.05$). There was no significant difference ($p>0.05$) in FSH levels when comparing the POI and NOF groups of women aged 31–36 years and the youngest group.

In women with PCOS, no correlation was identified between AMH concentration and age across all age categories examined ($p>0.05$). A similar result was obtained in the analysis of FSH, which indicated that there was no relationship between the concentration of this hormone and age ($p>0.05$) in women with PCOS. The obtained values of mean AMH and FSH levels in the three age groups according to the ovarian function status of POI, PCOS, and NOF are presented in Table 1.

Analysis of hormone concentrations in the NOF women group for each age category showed that AMH levels assumed the highest value in the 23–30 years group and decreased linearly with age. In contrast, FSH levels were higher in women aged 31–35 years compared to the younger group and then decreased slightly in the NOF 36–46 years group. AMH levels in women with POI, as in the control group, successively decreased with age, reaching the lowest level in the oldest group of subjects. The concentration of FSH in the group of women with POI showed some fluctuations, as its value decreased in the middle age range and then increased sharply in the 36–46 age range.

Table1. A comparative analysis of AMH and FSH concentrations between groups of women: POI vs. NOF and PCOS vs. NOF depending on age category

	Age	POI x ± SD	NOF x ± SD	PCOS x ± SD	P-Value	η2
AMH [ng/ml]	23–30	0.718 ± 0.20	2.425 ± 0.69	6.182 ± 3.49	NS [a]; <0.01 [b]	0.69
	31–35	0.638 ± 0.26	2.092 ± 0.62	6.762 ± 4.04	NS [a]; <0.01 [b]	0.71
	36–46	0.491 ± 0.32	1.855 ± 0.59	7.465 ± 9.81	NS [a]; <0.01 [b]	0.72
FSH [mlU/ml]	23–30	7.690 ± 2.13	6.480 ± 1.57	5.944 ± 1.65	NS [a]; NS [b]	–
	31–35	7.491 ± 1.98	7.510 ± 3.89	6.103 ± 1.61	NS [a]; <0.01 [b]	0.41
	36–34	10.965 ± 14.42	7.223 ± 2.12	6.000 ± 2.27	<005 [a]; NS [b]	0.33

Date expressed as mean ± standard deviation. p-Value was considered statistically significant. AMH: anti-Müllerian hormone, FSH: follicle stimulating hormone, POI: premature ovarian insufficiency, PCOS: PCOS-polycystic ovarian syndrome; NOF: normal ovarian function; a Comparison between POI and NOF, b Comparison between PCOS and NOF; NS: nonsignificantly different.

There was a significant difference in the comparison of AMH concentrations in women with PCOS and NOF according to age. Analysis of mean AMH concentration values in the NOF group by age range showed a tendency towards a physiological decrease in serum levels of the hormone, which was not confirmed in women with PCOS in the three age groups, where AMH values were increasing with age reaching the highest value in the oldest women ($p<0.01$). In the PCOS group, FSH levels remained constant in all age categories (5.94±1.65 – 6.10±1.61 mlU/ml) and did not differ with respect to mean FSH values in PCOS women in the youngest and oldest groups. Analysis showed a significant difference in FSH levels only in the 31–35 years category when comparing PCOS and NOF women ($p<0.01$).

Discussion

The results of this study show that the proportion of women with PCOS is increasing and POI is observed in increasingly younger patients. The observed trend of late motherhood is increasingly unsuccessful due to the depletion of the ovarian reserve. The pool of ovarian follicles formed during prenatal development represents the reproductive potential of a woman of reproductive age until completely exhausted.

A growing number of studies point to the high predictive value of AMH and its role in the management of ovarian follicle resources (Lambert-Messalian et al 2016; Pankhurst et al. 2017; Pankhurst et al. 2016; Pankhurst 2019; Urrutia et al. 2019; Sova et al. 2019).

Early preventive diagnosis is an essential tool for monitoring reproductive health. In the present study, the concentrations of the ovarian reserve markers AMH and FSH were assessed in women with POI and PCOS compared to the NOF group over age. The analysis in this study showed that the age of the patients is the primary determinant affecting reproductive potential. Progressive involution of the gonads and a decrease in their endocrine activity results in impaired functioning of the hypothalamic-pituitary-ovarian axis (Kaczmarek and Wolanski 2018, Lambalk et al. 2009).

Changes in AMH and FSH concentrations indicate an increase in the rate of ovarian follicle loss by reversing the ratio of growing to primordial follicles. The present study showed that AMH concentrations in women with NFO in the three age ranges 23–30,31–35 and 36–46 years differed significantly, although these findings were not supported by the FSH levels, where the expected upward trend in the oldest women was not observed. The results obtained from the AMH analysis were in line with the findings reported in other studies (Bragg et al. 2012, Pankhurst et al. 2016).

Studies still under-represent results with the age category, which determines physiological norms for AMH and other sex hormones (Hambridge et al. 2013).

The regression analysis presented in this paper indicates that there is a linear decrease in AMH levels with age, while FSH fluctuations are less pronounced, particularly in women with ovarian dysfunction (Kumar et al. 2010; Randolph et al. 2014).

Similar results were reported by Visser and Themmen (2014) where the high predictive value of AMH for ovarian reserve was confirmed. A study by Pawelczyk and team (2003) showed that age determines the increased risk of reproductive disorders, such as POI and PCOS. The AMH regression analysis conducted in this study showed that POI women 23–30 years of age had significantly lower ovarian reserve than the NOF group. The differences in AMH levels in POI women, compared to the control group in subsequent age ranges, was statistically nonsignificant, albeit marked. Pankhurst (2017) conceptualized the role of AMH-mediated regulatory mechanisms in the primary activation of ovarian follicles to preserve fertility for as long as possible. According to the researcher, women with high ovarian reserve have efficient mechanisms to inhibit follicle loss in the ovaries. In women with POI, low concentrations of AMH accelerate the recruitment of primary ovarian follicles, which improves reproductive fitness but over a relatively shorter period of time compared to women with normal ovarian function. This is the cost of maintaining fertility in women with POI. The concept presented by Pankhurst and team is supported by the results of the present study. The reduction in AMH levels and the decline in concentration with age indicate that the reproductive capacity period in POI will be shorter than that observed in women with NFO.

The results obtained from the analysis of AMH levels in women with POI are consistent with the study by Urrutia et al. (2019).

In women's POI, the relatively limited but fruitful reproductive period is followed by the cessation of ovarian function and the onset of premature menopause. AMH is the main factor modulating the process of inhibition in women of NOF or acceleration of follicle recruitment in the case of POI (Pankhrust 2017). The observed strategy promotes the optimization of the management of reproductive potential during a woman's reproductive period.

Further research is needed to clarify the relationship between AMH symptoms and PCOS. Hormonal stability in the ovarian follicle is determined by a number of factors related to the regulation of trophic transitions such as FSH. In order to more accurately examine ovarian activity in the present study, in addition to the AMH marker, a second indicator standardly used to assess ovarian activity – FSH. It remains negatively associated with AMH ($p<0.05$) (Pigny et al. 2003) which supports findings reported by Sowers et al. (2010). The researchers conducted an analysis of ovarian reserve marker concentrations in 20 irregularly menstruating women between 20 and 30 years of age, which did not show a physiological pattern of ovarian aging consistent with chronological age.

The present study showed that the sensitivity of the standard marker FSH was significantly lower than AMH in women with PCOS. The analysis of AMH levels in the PCOS group showed a high diagnostic value in all age categories indicating a significant difference compared with the control group. The results obtained are in line with the results of other researchers who recommend AMH as a diagnostic tool for PCOS (Ray et al. 2012, Vale-Fernandes et al. 2023, Meczekalski et al. 2016, Liu et al. 2022). The comparison of mean FSH levels only in women 31–35 years with PCOS and PFJ showed a statistically significant difference which supports the higher sensitivity of AMH as an indicator of ovarian reserve. Sova et al. (2019) also showed a negative correlation between AMH and FSH levels in a group of women with PCOS (n=319) and healthy women (n=96) where the mean age was 28.1 years. FSH levels were not significantly different between the PCOS and control groups. The mean values of AMH levels indicated a difference at a high level of significance in both study groups (Sova et al. 2019). This result is consistent with the results obtained in this study, where the study group was included in three age categories due to the determining effect of chronological age on the physiological norm of AMH and FSH concentrations in NOF women.

This methodology permitted a more precise examination of the fluctuations in mean AMH and FSH levels in a cohort of women with impaired ovarian function, including those with POI and PCOS, while controlling for age. The results obtained by Grossman et al. (2008) indicated an increase in AMH levels in women with PCOS in the study group at the age of 28–37 years, which was also confirmed by the analyses in the present study. The survey conducted in this study had several limitations. The study focused on women from two regions of Poland, despite the large sample size, it is worth expanding the project to other regions of the country. The study included several variables affecting AMH and women's reproductive status while future projects will require the inclusion of several other variables such as stress, lack of exercise, poor diet, overweight or diseases of the genitourinary system.

Conclusions

The findings of our study show that chronological age is a significant predictor of AMH levels and, as a result, of women's reproductive potential. These findings suggest that AMH is more responsive to age-related changes than the conventional ovarian reserve marker

FSH. Furthermore, the high predictive value of AMH for ovarian dysfunction was shown, particularly in the context of PCOS, where abnormalities were observed as early as age 23–30, while the FSH marker demonstrated insufficient sensitivity. Ovarian dysfunctions, such as POI and PCOS, can result in fertility issues. However, early diagnosis using anti-Müllerian hormone (AMH) testing can improve the likelihood of a successful pregnancy.

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Conflict of interests

The authors declare no potential conflicts of interest regarding the research, authorship, or publication of this article.

Authors' contribution

Both authors contributed equally to this manuscript.

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Anatomical alterations: biparietal thinning in antiquity. Review of published cases and a new case

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Abstract: Cranial anatomical variations, such as biparietal thinning, offer critical insights into the health and living conditions of ancient populations. Despite the presence of extensive archaeological records, biparietal thinning remains a relatively rare and understudied condition. This review aims to synthesize existing bioarchaeological literature on biparietal thinning, addressing its historical prevalence, geographical distribution, and potential etiologies.

This study integrates data from previous bioarchaeological research supplemented with a new case from skeletal remains excavated at the hypogeal cemetery of Santa Maria Maggiore in Vercelli, Northern Italy. The analysis included macroscopic examination, radiological imaging, and comparative analysis with clinical and paleopathological cases to identify and assess the characteristic features of biparietal thinning.

Our analysis of the skeletal remains of an old adult female individual revealed clear indicators of biparietal thinning. Notably, the thinning was bilateral, with the absence of diploe in the affected areas while maintaining the inner and outer tables of the cranial vault. These findings align with documented cases in the literature and contribute new data to the limited corpus of biparietal thinning cases.

This study underscores the importance of integrating paleopathological findings with modern medical knowledge to enhance the understanding of ancient diseases. The case from Vercelli provides an opportunity to explore the multifactorial origins of biparietal thinning and highlights the necessity of a multidisciplinary approach, combining archaeological, anthropological, and medical perspectives. By presenting this new case, we aim to stimulate further research into biparietal thinning and similar cranial pathologies, enriching the broader narrative of human health evolution.

Key words: parietal thinning, biparietal osteodystrophy, hypogeal cemetery, skeletal remains, bioarchaeology

Original article

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Introduction

The discovery of bilateral symmetrical thinning of parietal bones within skeletal remains in bioarchaeology is a rare occurrence. Despite the abundance of large skull collections from archaeological sites worldwide, only a handful of documented cases exist to date (Henneberg et al. 2002). Nonetheless, across epochs and continents, archaeological and anthropological investigations have unearthed cases of biparietal thinning among ancient human populations. In fact, this condition has been recognized for an extended period of time. Specifically, the earliest descriptions of parietal thinning were documented in the 18th century (Fusco et al. 2020). The prevalence of this condition, as reported by early studies such as Carriere in 1874, with estimated frequency of 0.4% in 1000 dry skulls, suggesting that this alteration is an uncommon occurrence in archaeological skeletal remains (Lodge 1975; Henneberg et al. 2002).

Geographically, archaeological occurrences have been sporadic, with reported cases spanning regions across Europe, including England, France, Italy, Austria, Romania and Spain, and other continents, such as America, Africa and Australia (Smith 1907; Durward 1929; Brothwell 1967; Lodge 1967; Dutta 1969; Arnaud and Arnaud 1976; Mallegni 1977; Bruyn 1978; Breitinger 1983; Ortner and Putschar 1985; Barnes 1994; Campillo 1996; Phillips 2007; Vargovà 2016; Fusco et al. 2020; Tonina et al. 2022; Vasile 2022). This widespread distribution implies that there is no association with any specific geographic region or ancestry (Mann et al. 2017).

The condition of parietal thinning is characterized by a distinct region of severe bone thinning located midway between the sagittal suture and the parietal prominence and typically presents symmetrically and bilaterally, rather than on one side only (Cvetković et al. 2022). The condition was first described in 1783 and has been known by various names over time, including *malum senile biparietale*, *senile biparietal atrophy*, biparietal thinning, and biparietal osteodystrophy, with the latter two being more commonly used in contemporary literature (Sandifort 1783; Bruyn 1978; Cvetković et al. 2022). Despite being an uncommon to rare condition in clinic, biparietal thinning is increasingly recognized as significant in forensic contexts. The presence of biparietal thinning can render an individual more susceptible to trauma, particularly if the site of impact involves the regions where the bone is thinnest within the depressions (Mann et al. 2017). Clinically, this condition is often discovered incidentally on imaging, patients may report bilateral scalp depressions, localized pain or sustain skull fractures resulting from falls or trauma (Tsutsumi et al. 2008; Yiu Luk et al. 2010; Sanati-Mehrizy et al. 2020; Yokota et al. 2024).

Modern clinical case records indicate that the majority of parietal thinning cases occur in individuals with a minimum average age of 50 for men and over 60 for women (Yılmaz et al. 2013; Mann et al. 2017; Fusco et al. 2020). Nevertheless, due to the scarcity of archaeological cases, it remains challenging to ascertain whether this condition was more prevalent in ancient populations compared to contemporary times (Henneberg et al. 2002).

Debate persists among researchers regarding the etiology of this phenomenon. Some attribute it to a developmental defect, while others associate it with progressive osteoporosis in the elderly (Ortner and Putschar 1985; Aufderheide and Rodríguez-Martín 1998). Other factor considered through the years, range from constant external pressure on the skull (often mentioned in archaeological studies of ancient Egyptians who wore heavy wigs) to syphilis, gonadal insufficiency and post-menopausal osteoporosis as it is more frequently observed in women (Smith 1907; Epstein 1953; Sanati-Mehrizy et al. 2020; Cvetković et al. 2022). Additionally, parietal thinning has been noted in multiple members of some families, but the hereditary aspect remains inconclusive (Camp and Nash 1994). However, the relationship between parietal thinning and age is notable, with both archaeological and clinical studies indicating a higher incidence in older individuals (Epstein 1953; Tsutsumi et al. 2008; Yiu Luk et al. 2010; Yılmaz et al. 2013; Mann et al. 2017; Fusco et al. 2020; Sanati-Mehrizy et al. 2020; Yokota et al. 2024).

As we investigate the complexities of this condition, numerous questions arise concerning its origins, prevalence and implications for ancient populations. Through the ongoing research and examination of archaeological remains, we aim to enhance the understanding of its significance in the broader context of human health and historical development.

In expanding the array of archaeological discoveries, we also present a new case of bilateral thinning of the parietal bones in a female individual unearthed from the subterranean burial site beneath the Church of Santa Maria Maggiore in Vercelli, Northern Italy, as part of a comprehensive review on this anatomical condition.

The co-cathedral of Santa Maria Maggiore was constructed in the 18th century under the direction of the Jesuits. It replaced the "*Santissima Trinità*" church and in 1780, it adopted the name of the former Santa Maria Maggiore Basilica that once stood nearby to the south. Underneath the church extends a hypogeal space, inaugurated in 1777 for funerary purposes, which reflects the architectural footprint of the overlying structure. This cemetery remained in use until the early 19th century, reflecting the continuity of funerary practices within this site (Tibaldeschi 1996; Destefanis et al. 2022; Fusco et al. 2023; Licata et al. 2023; Vanni et al. 2024).

Materials and Methods

Review

The aim of this review is to comprehensively summarize articles that discuss archaeological cases of parietal thinning available on major research platforms.

An initial search was conducted on PubMed using the following keywords: "Biparietal thinning", "Parietal thinning", "Bilateral parietal thinning", "Biparietal osteodystrophy", "Senile arthropathy", "Parietal osteodystrophy", "Biparietal senile disease" and "Malum senile biparietale". Each entry was initially reviewed through its title and abstract, with further examination of the full text if the term's usage was unclear. Articles focusing on skeletal biology from an archaeological, anthropological and paleopathological perspective were included. On the other hand, articles that were not authored in English, and that addressed modern clinical and forensic cases, were excluded. The online search was conducted with no limitations on publication date and geographical area.

Subsequently, a secondary search was conducted on Scopus, Google Scholar and Research Gate using the same keywords and inclusion/exclusion criteria.

Case report

The new case of biparietal thinning, herein presented, originates from the ongoing bioarchaeological investigation at the Church of Santa Maria Maggiore in Vercelli, Piedmont, Northern Italy (Fig. 1.A).

As previously stated, beneath the Church is located a hypogeal cemetery, which was used both for new interments and the relocation of remains from the former complex (Tibaldeschi 1996; Fusco et al. 2023; Vanni et al. 2024).

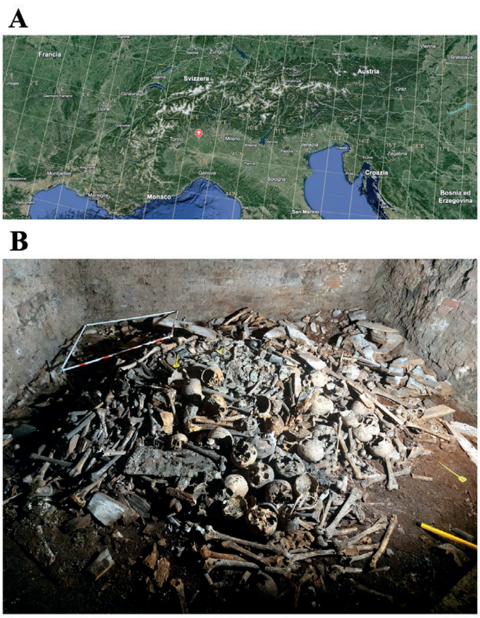

Figure 1. A) Map of Northern Italy highlighting the location of Vercelli; B) The hypogeal chamber, "Hypogeum 1".

This vaulted subterranean space contains two underground ossuaries. Bioarchaeological investigations are currently underway in the first ossuary, known as 'Hypogeum 1' (Fig. 1.B). It has been divided into square sectors labeled with a combination of numbers (1 to 6) and letters (A to F), and each sector is further subdivided into four corners (North-East, North-West, South-East, South-West) (Fig. 2). This system facilitates the identification of the original location of recovered bones, enabling a more systematic analysis (Orsi et al. 2024).

The cranium featured in this case report was recovered during the early stages of the archaeological investigation. Specifically, it was found in quadrant 1C, Stratigraphic Unit 1, South-East corner (VC SMM24 1C SU1 SE).

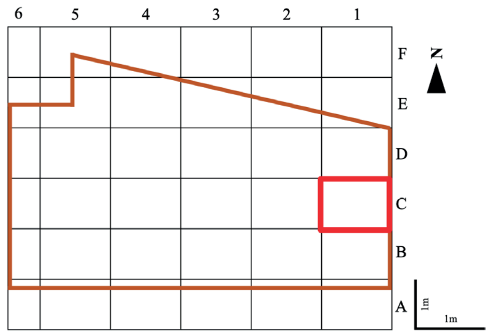

Figure 2. Subdivision of the hypogeum into quadrants with the highlighted square indicating the quadrant (1C) in which the skeletal remain was recovered

To reconstruct the biological profile of the individual, an anthropological analysis was conducted. Sex was determined by observing the diagnostic morphological features of the skull (Buikstra 1994). Age at death was estimated by analyzing the degree of cranial suture closure (Meindl and Lovejoy 1985) and the dental wear (Lovejoy 1985). All available bone measurements were conducted using an electronic digital caliper. Furthermore, a detailed examination of the cranium was performed to identify any pathological conditions. This included macroscopic observations conducted both through unaided visual inspection and with the aid of a magnifying glass. A CT scan was conducted using a Canon

Aquilion Start machine with 16/32 slices, following a protocol of 150 mA, 1-second rotation, and 120 kV, with a slice thickness of 0.2 mm. To assess the changes in bone morphology and to measure the bone thickness, the CT scans were analyzed using the Weasis DICOM medical viewer software, version number 4.5.1.0.

Results

Review

The literature review conducted on cases of archaeological biparietal thinning has highlighted several significant findings. The online search across all keywords and search platforms yielded a total of 90.153 hits. The strict selection criteria applied during the review process, focusing exclusively on bioarchaeological and paleopathological contexts, led to the exclusion of most documents, which were predominantly clinical, forensic, or duplicates across platforms. Furthermore, a significant number of articles were excluded due to a lack of relevance to the topic. In addition, several documents were excluded because they were not published in English and not available online, such as Carimati (1954), Piccoli (1965), Kharon and Lifshits (1969) and Takunyacıoğlu (2019). For completeness, in Table 1, we have reported the nine bioarchaeological cases of biparietal thinning that were excluded from the review according to the exclusion criteria (Arnaud and Arnaud 1976; Mallegni 1977; Breitinger 1983; Günay 2005 cited in Çırak 2009; Baggieri 2007; Sağır 2009; Çırak et alt. 2021; Kokotović 2021; Vasile 2022).

Out of all the hits examined through the various search terms and the different search engines, excluding overlapping data (i.e., the same results for different keywords and research platform), only 26 articles (Smith 1907; Durward 1929; Brothwell 1967; Dutta 1969; Bruyn 1978; David 1979; Bartell 1994; Mallegni and Severini 1997; Henneberg et al. 2002; Strouhal et al. 2003; Mulhern 2005; Phillips 2007; Willems et al. 2009; Malnasi 2010; Milsom 2012; Komáry and Fóthi 2013; Seiler and Rühli 2014; Onderka et al. 2015; Vargová et al. 2016; Fusco et al. 2020; Lockwood et al. 2020; Smith et al. 2020; Kozieradzka-Ogunmakin et al. 2021; Tonina et al. 2022; Tornberg 2022; Waziri et al. 2023; Kotze et al. 2024) met the inclusion criteria and therefore they have been included in this review. This corresponds to an exclusion rate of 99.97%, which highlights the rarity of biparietal thinning cases in the archaeological and paleopathological literature available online.

Table 1. Cases excluded from the review

Site	Chronology	Sex	Age	Diagnosis	Diagnostic method	Literature	Reason for exclusion
Ancient Paleocristian Cemetery of the chapel Sancta-Maria de Olivia à Beaulieu	/	M	50+	Biparietal thinning	Macroscopic and radiographic analysis	Arnaud and Arnaud 1976	Not authored in English
Eneolithic necropolis of the Gaudo (Paestum), Italy	Eneolithic	F	50	Biparietal thinning	Macroscopic and radiographic analysis	Mallegni 1977	Not authored in English
Great pyramid of Cheops	Ancient Egypt	3 F, 1 M	30–60	4 skulls presenting biparietal thinning	Macroscopic analysis	Breitinger 1983	Not authored in English

Table 1. (cont.)

Site	Chronology	Sex	Age	Diagnosis	Diagnostic method	Literature	Reason for exclusion
Kelenderis, Anatolia	19th century	M	60	Probable case of biparietal thinning	Macroscopic analysis	Günay 2005 cited in Çırak 2009	Not authored in English
Church of San Martino di Ovaro, Udine, Italy	Late Middle Ages	F	33–49	Biparietal thinning	Macroscopic and radiographic analysis	Baggieri 2007	Not authored in English
Karaca Ahmet cemetery, Istanbul	Ottoman period	F	55–60	Biparietal thinning	Macroscopic analysis	Sağır 2009	Not authored in English
Balatlar Church population in Sinop, Anatolia	18th century	M	Older	Biparietal thinning	Macroscopic and radiographic analysis	Çırak et al. 2021	Not authored in English
Mukoše site near Goriš moder Dalmatia	16th century	M	/	Biparietal thinning	Macroscopic analysis	Kokotović 2021	Not authored in English
Middle Byzantine necropolis from Noviodunum-Isaccea Așezare Civilă Est (Tulcea County, Dobruja, Romania)	Medieval Age (11th–13th centuries)	M	42	Biparietal thinning	Macroscopic analysis	Vasile 2022	Not authored in English

Case report

The analysis of the examined cranium, VC SMM24 1C SU1 SE, revealed several notable characteristics (Fig. 3). The neurocranium is largely intact but missing substantial portions of the left parietal and occipital bones, likely due to taphonomic processes.

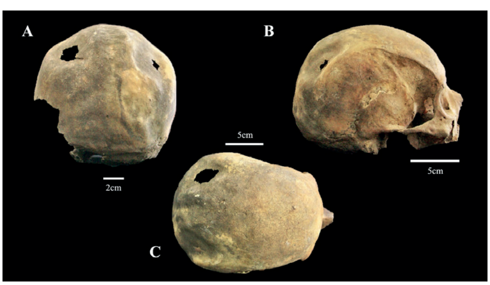

Figure 3. Views of the individual A) posterior view; B) lateral view; C) superior view

The subject was identified as an old adult female (Buikstra 1994), as suggested by the complete ante mortem loss of teeth with significant alveolar resorption and the complete obliteration of all ectocranial sutures.

More detailed observations identified two circular areas of reduced thickness in the cranial table in the upper parietal region, located symmetrically on both sides of the sagittal suture. These areas of biparietal atrophy have the following dimensions: the left area measures 4.65 cm in transverse diameter and 3.62 cm in sagittal diameter while the right area measures 4.47 cm in transverse diameter and 3.1 cm in sagittal diameter. The depth of the thin area on the right side of the skull is 0.56 cm, whereas the depth on the left side cannot be measured due to a central taphonomic hole provoked by the extreme thinness, and therefore delicacy, of the area.

CT images showed bilateral and nearly symmetrical thinning involving the posterior sections of the parietal bones. Furthermore, in the areas unaffected by parietal thinning, the inner and outer tables, along with the diploic space between them, were clearly distinguishable and showed no anomalies. In the transitional zone, a reduction in bone thickness was observed, with the diploe appearing thinner compared to normal bone. Finally, in areas where the trabecular bone was completely resorbed, the inner and outer tables merged to form a thin layer of cortical bone (Fig. 4). The normal cranial bone thickness in unaffected parietal sections measured 5.4 mm, 4.3 mm, and 3.5 mm. In contrast, the thickness in the two resorbed areas was reduced, measuring 2.5 mm, 1.9 mm, and 1.7 mm on the right side, and 3.1 mm, 2.6 mm, and 2.4 mm on the left side.

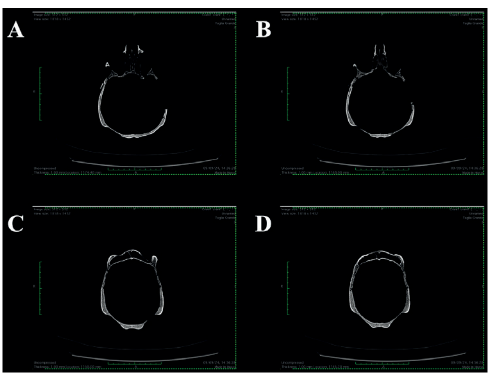

Figure 4. CT scan of individual in the axial plane, coronal vision. A) Extreme thinning is noted in the right parietal bone, with the absence of diploe and the fusion of the outer and inner tables; B) Progressive thinning of the parietals, with the junction of the outer and inner tables also noted in the left parietal bone; C) Progressive thinning of the parietals, with a taphonomic hole visible in the left parietal bone; D) Stage of thinning in which progressive absence of diploe and fusion of the outer and inner cortices is noted in the right parietal bone

Discussion

The examination of biparietal thinning in skeletal remains offers significant insights into cranial anatomical variations and provides a broader perspective on historical health. This review, incorporating the newly presented case from the Church of Santa Maria Maggiore in Vercelli, aims to contribute to the understanding of this rare condition.

The literature review highlights the rarity and distribution of biparietal thinning across various historical periods and locations (Smith 1907; Durward 1929; Brothwell 1967; Lodge 1967; Dutta 1969; Arnaud and Arnaud 1976; Mallegni 1977; Bruyn 1978; Breitinger 1983; Ortner and Putschar 1985; Barnes 1994; Campillo 1996; Phillips 2007; Vargovà 2016; Fusco et al. 2020; Tonina et al. 2022). Analysis of reviewed paleopathological cases suggests that although biparietal thinning is infrequent in archaeological settings, it is manifested across a range of populations (Henneberg et al. 2002) (Tab. 2), indicating no clear association with any specific geographic region or ancestry (Mann et al. 2017).

The recently discovered case presented here provides new insights into biparietal thinning. The individual exhibits classic morphological features of parietal thinning such as symmetrical depressions of the parietal bones characterized by a significant thinness of the area (Cvetković et al. 2022), enriching the existing bioarchaeological cases. Notably, this case offers valuable data to increase the understanding of biparietal thinning, particularly in expanding the geographical documentation of this condition within Europe.

Table 2. Archaeological reviewed cases of parietal thinning

Site	Chronology	Sex	Age (years)	Diagnosis	Diagnostic method	Literature
Collection of ancient Egyptian crania	Ancient Egypt, period between the 4th and 19th dynasties	/	/	Cranial thinning	/	Smith GE 1907
Skull collection of the Department of Anatomy, University of Otago, New Zealand (Maori an Moriori, Chinese)	/	3 M, 2 ND	25–74	5 skulls presenting biparietal thinning	Macroscopic analysis	Durward A 1929
/	Early Britain	/	/	Biparietal thinning	/	Brothwell 1967
Harappa, West Punjab India	2300 B.C, Bronze Age	F	45	Biparietal thinning	Macroscopic analysis	Dutta 1969
Neurological collection, Department of Neuropathology, University Hospital, Leiden, The Netherlands	/	7 F, 1 M	51–86	8 cases of biparietal thinning	Macroscopic, X-ray and histological analysis	Bruyn1978
Manchester Museum	Ancient Egypt	M	Elderly	Biparietal thinning	Radiographic analysis	David 1979
Naqada, Egypt	Predynastic	/	Middle to older adults	6 skulls presenting biparietal thinning	Macroscopic analysis	Bartell 1994
Necropolises of Cantone (Collelongo) and Arciprete (Trasacco), Italy	Late Roman Age (1st century B.C., 1st century A.D.)	M	Old adult	Biparietal thinning	Macroscopic and X-ray analysis	Mallegni and Severini 1997
Santa Venera Necropolis of Ancient Poseidonia, Italy	4th–5th century B.C.	F	>70	Biparietal thinning	Macroscopic analysis	Henneberg et al. 2002
Abusir, Egypt. Shaft tomb of "Iufaa"	Late 26th dynasty (before 525 B.C.)	M	25–30	Biparietal thinning	Macroscopic, radiographic and histological analysis	Strouhal et al. 2003
Western Cemetery at Giza,Egypt	5th dynasty	M	27–52	Biparietal thinning	Macroscopic analysis	Mulhern 2005
Egypt (crania from various museum collections)	Ancient Egypt	/	/	54 cases of parietal thinning	Macroscopic analysis and CT scan	Phillips 2007
Deir al-Barsha, Egypt	Ancient Egypt (Old Kingdom)	1 F, 1 M, 1 ND	>30–35	3 cases of biparietal thinning	Macroscopic analysis	Willems et al. 2009

Site	Chronology	Sex	Age (years)	Diagnosis	Diagnostic method	Literature
Dayr Al-barsha And Sheikh Said, Egypt	Ancient Egypt	3 F, 6 M, 1 ND	>35	10 cases of biparietal thinning	Macroscopic analysis	Malnasi 2010
Southern Saskatchewan, Canada (Adamiak cranium)	Middle and Late Middle Precontact Periods	F	35–45	Biparietal thinning	Macroscopic analysis	Milsom, 2012
Material of el-Assasif, Thebes (inv. no. 7357 and 7358) from the Anthropological Collection of the Natural History Museum of Paris	Ancient Egypt, 18th Dynasty	1 M (inv. no. 7357), 1 F (inv. no. 7358)	Senile	Biparietal thinning (inv. no. 7357) and left parietal thinning (inv. no. 7358)	Macroscopic analysis	Komáry and Fóthi 2013
Geneva Museum of Art and History, Switzerland	Third intermediate period, Thebes, Egypt	F	40–50	Biparietal thinning	Macroscopic and radiographic analysis	Seiler and Rühli 2014
Náprstek Museum, Prague	Third Intermediate Period, Egypt most likely Western Thebes	F	40–50	Biparietal thinning	Macroscopic and radiographic analysis	Onderka et al. 2015
Cemetery Prague-Zličín	Migration Period (5th century)	F	45–50	Right parietal thinning	Macroscopic analysis	Vargová et al. 2016
Cairo, Egypt	Ancient Egypt	M	Elderly	Biparietal thinning	X-ray and CT scan	Fusco et al. 2020
Maidstone Museum (Kent, United Kingdom)	2700-year-old mummy "Ta-Kush"	F	>40	Biparietal thinning	Microcomputed-Tomography	Lockwood et al. 2020; Smith et al. 2020
Theban rock-cut Tombs at Sheikh 'Abd al-Qurna. Tomb TT95	Ancient Egypt	M	>45	Biparietal thinning	Macroscopic and X-ray analysis	Koziera-dzka-Ogunmakin et al. 2021
Medieval cemetery of Caravate (north Italy)	Medieval Age	F	45–55	Biparietal thinning	Macroscopic, radiographic and histological analysis	Tonina et al. 2022
Kyhl bjersbacken, Vellinge parish, Scania	Neolithic-Early Bronze Age	F	>60	Biparietal thinning	Macroscopic analysis	Tornberg 2022
Rock cut tomb of Penmes at Saqqara, Egypt	Old Kingdom	F	>60	Biparietal thinning	Macroscopic analysis	Waziri et al. 2023

Table 1. (cont.)

Site	Chronology	Sex	Age (years)	Diagnosis	Diagnostic method	Literature
South Africa (San people)	19th century (1875)	F	45–60	Biparietal thinning	Macroscopic analysis and CT scan	Kotze et al. 2024

Descriptions of parietal thinning date back to the 18th century, with the condition referred to by various names such as "biparietal senile disease," "senile arthropathy," "biparietal thinning" and "parietal osteodystrophy" (Sandifort 1783; Bruyn 1978; Cvetković et al. 2022). Historically, this condition typically involves the posterior parasagittal regions and presents with an oval or quadrilateral flat shape (Fusco et al. 2020). Cederlund's 1982 study provided a radiological classification of parietal thinning into three stages: (i) superficial thinning visible as a radiolucent area on tomographic images; (ii) considerable thinning with more than half of the bone substance lost while the diploe remains; and (iii) complete loss of the diploe and external surface. This classification helps to understand the progression of the condition, which initially affects only the outer table, leaving the inner table intact (Cederlund 1982). Bruyn and Bots described two primary forms of biparietal thinning: flat and grooved. The flat type is more commonly documented and features a plane-like depression, while the grooved type features a crater-like depression, typically oval in shape (Bruyn 1978). Reaching a differential diagnosis in our case is challenging; we can only note the presence of bilateral parietal thinning that, according to Cederlund's classification, can be classified as third-degree bilateral parietal thinning (Cederlund 1982). Furthermore, based on Bruyn's classification, our case can be identified as the flat type (Bruyn 1978).

Computer tomography typically shows thinning or absence of the lamina corticalis externa, loss of the diploe, and preservation of the lamina corticalis interna (Tonina et al. 2022).

The CT findings of bilateral symmetrical thinning in the posterior sections of the parietal bones align with previously reported cases in both clinical and archaeological literature. A similar pattern was observed in the clinical case reported by Yiu Luk et al. (2010), where computed tomography revealed bilateral and symmetrical parietal bone thinning, primarily attributed to the loss of diploe and thinning of the outer table. This case shares notable similarities with our findings, particularly in the bilateral presentation and the thinning of the diploe.

In the bioarchaeological field, Tonina et al. (2022) documented a more severe progression of bone thinning, where radiological images demonstrated thinning of the diploe to the extent that it reached the inner table, with a complete absence of the outer table. While our finding also shows diploic resorption, the presence of both cortical tables in the affected areas of our case differentiates it from the more extreme thinning observed by authors.

Fusco et al. (2020) similarly reported a case of biparietal thinning, where the external cranial table appeared particularly affected, showing significant reduction and resorption, nearly exposing the diploe. Our findings deviate from this observation, as the affected areas featured the complete resorption of the trabecular bone, showing a thin cortical layer formed by the merging of the inner and outer tables.

Kotze et al. (2024) reported severe calvaria thinning characterized by large depressions, attributed to extreme resorption of the diploe, which led to diminution of both the outer and inner tables. Despite the similarity of presentation with our case, Kotze's findings demonstrated a more extreme loss of bone integrity, underscoring the variability of biparietal thinning presentations.

In a forensic case, Cvetković et al. (2022) described a progressive thinning of the outer table, which eventually merged with the inner table, leaving the inner table relatively unaffected. This pattern bears striking resemblance to the resorptive process observed in our CT scans, where the trabecular bone loss resulted in the merging of the cortical layers, though our findings also indicate that the diploe in the transitional zones remained somewhat visible, albeit thinner than in unaffected areas.

These comparative cases highlight the variable nature of biparietal thinning across different contexts, whether clinical, forensic, or paleopathological, but also underscore a common theme: the thinning process often involves selective loss of diploic bone, with varying degrees of involvement of the outer and inner cortical tables. The extent and progression of the thinning, as well as the involvement of one or both tables, appear to differ based on individual cases, suggesting variability in the progression and underlying causes of biparietal thinning.

In addition, the results of various bioarchaeological studies highlight differences in the dimensions of biparietal atrophy areas, with some cases showing greater degree of thinning in the right parietal region (Mallegni 1977; Seiler 2014; Fusco et al. 2020; Tonina et al. 2022). In our case, however, it is challenging to make a precise assessment due to taphonomic damage present in the left area of thinning. These dimensional variations may reflect different stages of disease progression, underscoring the need for further research to fully understand the clinical and prognostic implications associated with such differences.

The debate surrounding the causes of biparietal thinning remains unresolved, with theories ranging from developmental defects and osteoporotic conditions to external pressures and genetic predispositions (Smith 1907; Epstein 1953; Cvetković et al. 2022). The case from Vercelli, involving an old adult female, aligns with contemporary clinical observations indicating a higher prevalence in older individuals and in the female gender (Epstein 1953; Dutts 1969; Mann et al. 2017). This correlation supports the hypothesis that age-related bone changes could be a significant contributing factor. Indeed, this condition appears to be associated with the reduction or cessation of sex hormone activity (Epstein 1953). Furthermore, hormonal imbalances can lead to osteoporosis, which is also linked to a deficiency of osteoclasts, a condition found in histopathological analyses in some cases of parietal thinning (Schmidt 1937; Yiu Luk et al. 2010; Sanati-Mehrizy et al. 2020). However, the variability in the proposed causes and the widespread geographical-historical distribution of occurrences, underscores the necessity for further research to clarify the precise etiology in historical contexts.

Documenting biparietal thinning in historical populations enhances the broader comprehension of ancient health conditions. By integrating data from various archaeological sites, such as Vercelli, researchers can discern patterns and potential causes of this condition more effectively. Continued study of such cases not only sheds light on specific pathological conditions but also augments the overall knowledge of ancient human health and disease.

The ongoing challenge lies in further elucidating the causes and implications of biparietal thinning. Future research should prioritize the collection of additional cases from diverse geographical and temporal contexts to identify common factors. Employing advanced imaging techniques and adopting multidisciplinary approaches, incorporating archaeology, anthropology, and medical science, will be crucial in unraveling the complexities of this condition. Furthermore, exploring genetic and environmental factors in conjunction with skeletal evidence could yield a more nuanced understanding of biparietal thinning.

Conclusions

The discovery of a new case from the Church of Santa Maria Maggiore in Vercelli enhances our knowledge of this rare condition. The case study showing typical signs of biparietal thinning, reinforces contemporary clinical observations of its increased prevalence among older populations and the female sex. This alignment suggests that age-related bone changes may play a crucial role in the development of this condition.

However, the diverse hypotheses regarding its causes, ranging from genetic and environmental factors to external pressures, reveal the complexity of biparietal thinning indicating that multiple factors are likely involved. The geographical diversity of documented cases suggests that biparietal thinning was not restricted to any specific population or period. This widespread occurrence points to a multifaceted etiology that warrants further interdisciplinary investigation.

Future research should focus on broadening the scope of paleopathological studies and integrating new methodologies to deepen our understanding of biparietal thinning. By examining a wider range of cases and employing advanced imaging and analytical techniques, researchers can uncover the most probable causes and impacts of this condition. Such efforts will not only advance our knowledge of ancient human health but also contribute to a more nuanced understanding of the factors influencing skeletal pathology across different historical and geographical contexts.

In conclusion, the insights gained from the Vercelli case study and the broader review highlight the importance of continued research into biparietal thinning. Expanding bioarchaeological research and integrating new findings are essential for unraveling the complexities of this rare condition and understanding its implications for ancient human health.

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Authors' contribution

NR: Conceptualization, Investigation, Writing - original draft; RF: Conceptualization, Writing – review & editing; AV: Data Curation, Writing - review & editing; FG: Resources, Visualization, Writing - review & editing focused on archaeological data; AM: Resources, Visualization, Writing - review & editing; ML: Project administrator, Supervision, Writing - review & editing

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