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Value of digit ratio 2D:4D, a biomarker of prenatal hormone exposure, is stable across the menstrual cycle

Magdalena Klimek[a,*], Urszula M. Marcinkowska[a], Grazyna Jasienska[a]

[a] Department of Environmental Health, Faculty of Health Sciences, Jagiellonian University Medical College, Grzegorzecka 20, 31–531 Krakow, Poland

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ABSTRACT

Digit ratio (2D:4D) is used as a marker of prenatal hormone exposure and, consequently, as a predictor of many characteristics throughout a woman's lifespan. A previous study has suggested that values of 2D:4D vary across menstrual cycles and further questioned the reliability of a single measurement of 2D:4D among cycling women, while another study failed to confirm these results. However, these studies estimated the timing of cycle phases based on a date of menstruation reported by participants and also had small sample sizes. For our study, we evaluated potential changes in 2D:4D values across a menstrual cycle in a group of women among whom the phases of the menstrual cycle were determined by hormonal (luteinizing hormone based) ovulation tests. We studied 32 naturally cycling women aged 22–37 from rural Poland. Lengths of second and fourth digits were measured based on scans of both hands taken three times (i.e. in the follicular phase, peri-ovulatory phase and luteal phase of the cycle) for each participant. No differences in 2D:4D value across the menstrual cycle were detected either when right-hand, left-hand, and mean 2D:4D for both hands were analysed, nor when difference in the 2D:4D value between hands ($D_{left\text{-}right}$) was evaluated. We documented that 2D:4D is independent of the phase of the menstrual cycle and these findings suggest that among naturally cycling women, a value of 2D:4D can be reliably obtained from measurements taken during any day of the menstrual cycle.

1. Introduction

Female soft-tissue characteristics might change in their volume and dimensions depending on the phase of the menstrual cycle [1]. Flow of body fluids (i.e. soft-tissue hydration and transepidermal loss of water or retention), which depends on fluctuating hormone levels over the course of the menstrual cycle, are one of the possible mechanisms explaining observed changes [2]. It has been suggested that bilateral soft tissue traits (i.e. breast, fingers, and ears) have higher asymmetry at the beginning and end of the menstrual cycle, compared to the peri-ovulatory phase [1]. Similarly, a woman's level of facial [3,4,5] and overall body fluctuating asymmetry [6] also depends on fertility status.

Digit ratio (2D:4D) is a biomarker of prenatal hormone exposure and correlates with multiple traits in post-natal life (i.e. [7,8,9,10]). If finger lengths are labile throughout the menstrual cycle it can be expected that values of 2D:4D, which is based on finger measurements, might also show fluctuations depending on the phase of the cycle. Two previous studies have investigated potential changes in this biomarker between menstrual phases. One study observed a difference in 2D:4D value across the menstrual cycle [11], while the other did not [12]. However, both studies were based on very small groups of participants: 13 naturally cycling women and 6 women using oral contraceptives [11] in the first, and 12 naturally cycling women [12] in the second. More importantly, in both studies, the phases of the menstrual cycle were identified based on an interview during which a date of menstruation was given by participants.

For our study, we tested the potential changes in finger lengths and 2D:4D value across the menstrual cycle in a larger than previously studied (but still relatively limited in number) group of women, among whom phases of the menstrual cycle were determined by ovulation (LH strip) tests.

2. Materials and methods

Thirty-two women aged 22–37 from the Mogielica Human Ecology Study Site located in rural southern Poland [13], who were neither pregnant, breastfeeding nor taking hormonal contraceptives for at least three months prior to participation, were included in the study. Each participant was visited at home three times during one menstrual cycle in order to take hand scans. Phases of the menstrual cycle were determined by self-performed ovulation strip tests based on luteinizing hormone levels (LH tests). The women conducted LH tests from the

* Corresponding author.
E-mail addresses: mklimek@cm-uj.krakow.pl (M. Klimek), urszula.marcinkowska@uj.edu.pl (U.M. Marcinkowska), jasienska@post.harvard.edu (G. Jasienska).

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10th until the 20th days of their cycles or until a test indicated a positive result. Luteinizing tests have been proved to be highly accurate in detecting timing of ovulation [14]. Visits were scheduled in the early follicular phase (between 2nd and 8th day), at the peri-ovulatory phase (not later than 72 h after a positive result of the ovulation test or if the test did not indicate an LH surge, on the 20th day of the cycle), and in the mid-luteal phase (about one week after ovulation). Twenty-two out of thirty-two women had a positive result from the LH test. Mean day of ovulation was − 14.9 days (SD = 2.15) before the onset of the next menses and the range of ovulation day varied between − 10th and − 22nd day of the cycle.

Right and left hand scans were taken three times for each woman during their menstrual cycle (in the follicular, peri-ovulatory, and luteal phase) with a Canon CanoScan LiDE 110 device. All participants were instructed to place their hands flat and not to push on the scanner's surface. None of the participants reported any injuries or diseases that could influence finger lengths. An experienced researcher measured each scanned finger twice and measurements were repeated by another experienced researcher. Measurements were performed with an accuracy of within 0.1 mm using GIMP 2.8 Software, according to previously published procedures. This type of measurement method was chosen since it has been suggested that finger length measurements based on scans are more reliable than direct measurements, photocopies or printed scans [15]. Digit ratio 2D:4D was calculated for each hand and for each phase of the menstrual cycle as a mean of measurements performed by both observers. The study was approved by the Jagiellonian University Bioethical Committee.

2.1. Statistical analyses

Tested parameters (across three phases of the menstrual cycle) were: (i) differences in finger lengths (second and fourth fingers on both hands separately), (ii) differences in 2D:4D value in right-hand and left-hand, (iii) differences in mean 2D:4D value for both hands, and (iv) difference in 2D:4D between hands ($D_{left\text{-}right}$), calculated as R2D:4D − L2D:4D (Table 1). Differences were analysed by repeated measures ANOVA using Statistica 12 Software.

2.2. Interobserver and intraobserver reliability

To avoid possible inter- and intra-observer error, both observers performed measurements twice for each participant at a few weeks interval and were blinded to the identity of the participants. To test the reliability of the measurements, Interclass Correlation Coefficient (ICC) with two-way mixed-effects model in absolute-agreement type [16] was calculated using IBM SPSS Statistics Software version 24. The ICC for Observer 1 varied between 0.94 and 0.99 and for Observer 2 between 0.90 and 0.98. The ICC between the two Observers varied between 0.89 and 1.00. The reliability analysis showed excellent intra- and inter-observer agreement.

3. Results

Digit ratio (2D:4D) values did not differ among the three phases of the menstrual cycle when calculated for right ($p = 0.48$) or left hand ($p = 0.46$). The results remained insignificant when 2D:4D was calculated as a mean value of both hands ($p = 0.70$) or as the difference between both 2D:4D's ($D_{left\text{-}right}$) ($p = 0.34$) (Fig. 1).

When lengths of fingers among cycle phases were compared, we did not observe a statistically significant difference in finger lengths among the three phases for second finger on the right hand ($p = 0.17$) or second finger on the left hand ($p = 0.90$), however, we did observe a difference in the lengths of the fourth finger on the right hand ($p = 0.01$) and a difference approaching borderline significance in the fourth finger on the left hand ($p = 0.06$) (Fig. 1). Post hoc comparisons (Bonferroni test) revealed a significant difference in fourth finger length on the right hand between the peri-ovulatory phase and luteal phase ($p = 0.03$) and the follicular phase and luteal phase ($p = 0.04$). The analyses were then repeated for women who had a positive result of the LH test ($n = 22$). In this group with confirmed ovulation, values of 2D:4D did not change among the three phases of the cycle for left-hand 2D:4D ($p = 0.63$), right-hand 2D:4D ($p = 0.37$), mean 2D:4D for both hands ($p = 0.49$), and difference in 2D:4D between hands ($D_{left\text{-}right}$) ($p = 0.59$). Similarly, lengths of the second finger ($p = 0.98$) and the fourth finger ($p = 0.21$) on the left hand, as well as the lengths of second ($p = 0.63$) and fourth finger ($p = 0.12$) on the right hand, did not differ significantly.

4. Discussion

In this study, value of 2D:4D biomarker was stable across follicular, peri-ovulatory, and luteal phases in a group of rural Polish women who performed LH ovulation tests to determine phases of their menstrual cycle. Despite the fact that we observed a difference in the length of fourth finger on the right hand, this did not influence the overall 2D:4D value (for the right hand, left hand, and mean for both hands), including any difference in 2D:4D between hands ($D_{left\text{-}right}$), considered as a pointer of bilateral asymmetry. When the study group was limited only to women who had a positive LH test, no significant differences in values of 2D:4D, or in lengths of fingers, between phases of the menstrual cycle were observed.

It is important to mention at this point that finger length measurement methodology might play an important role and direct vs indirect measurements may give different 2D:4D values [17]. Finger bone lengths are unchangeable but volume of soft tissue at the top of the finger might change during the cycle [1]. Therefore, if the measurement is performed with a manual caliper (i.e. soft tissue at the top of the finger is squeezed) it may lead to different results when compared to the measurements performed using computer-based methods (i.e. from hand scans or photocopies analysed in computer programs), as previously discussed by Allaway [16]. Further studies should, therefore,

Table 1
Descriptive statistics of studied parameters across the phases of the menstrual cycle and the results of repeated measures ANOVA analyses.

		Follicular phase	Peri-ovulatory phase	Luteal phase	ANOVA	
		Mean (SD)	Mean (SD)	Mean (SD)	F	*p*-Value
Right hand	2nd finger	69.65 (3.939)	69.65 (3.802)	69.84 (3.899)	$F_{(2,56)} = 1.81$	0.17
	4th finger	71.48 (3.869)	71.47 (3.816)	71.85 (3.893)	$F_{(2,56)} = 4.46$	**0.01**
Left hand	2nd finger	68.80 (3.761)	68.74 (3.797)	68.81 (3.713)	$F_{(2,56)} = 0.09$	0.91
	4th finger	71.33 (3.903)	70.99 (3.506)	71.02 (3.718)	$F_{(2,56)} = 3.03$	0.06
Right hand 2D:4D		0.97 (0.020)	0.97 (0.021)	0.97 (0.021)	$F_{(2,56)} = 0.74$	0.48
Left hand 2D:4D		0.97 (0.025)	0.96 (0.031)	0.97 (0.029)	$F_{(2,56)} = 0.78$	0.46
Mean of right and left hand 2D:4D		0.97 (0.023)	0.97 (0.021)	0.97 (0.022)	$F_{(2,56)} = 0.36$	0.70
$D_{left\text{-}right}$		− 0.006 (0.0223)	− 0.009 (0.0270)	− 0.003 (0.0238)	$F_{(2,56)} = 1.10$	0.34

Bold numbers indicate statistically significant result.

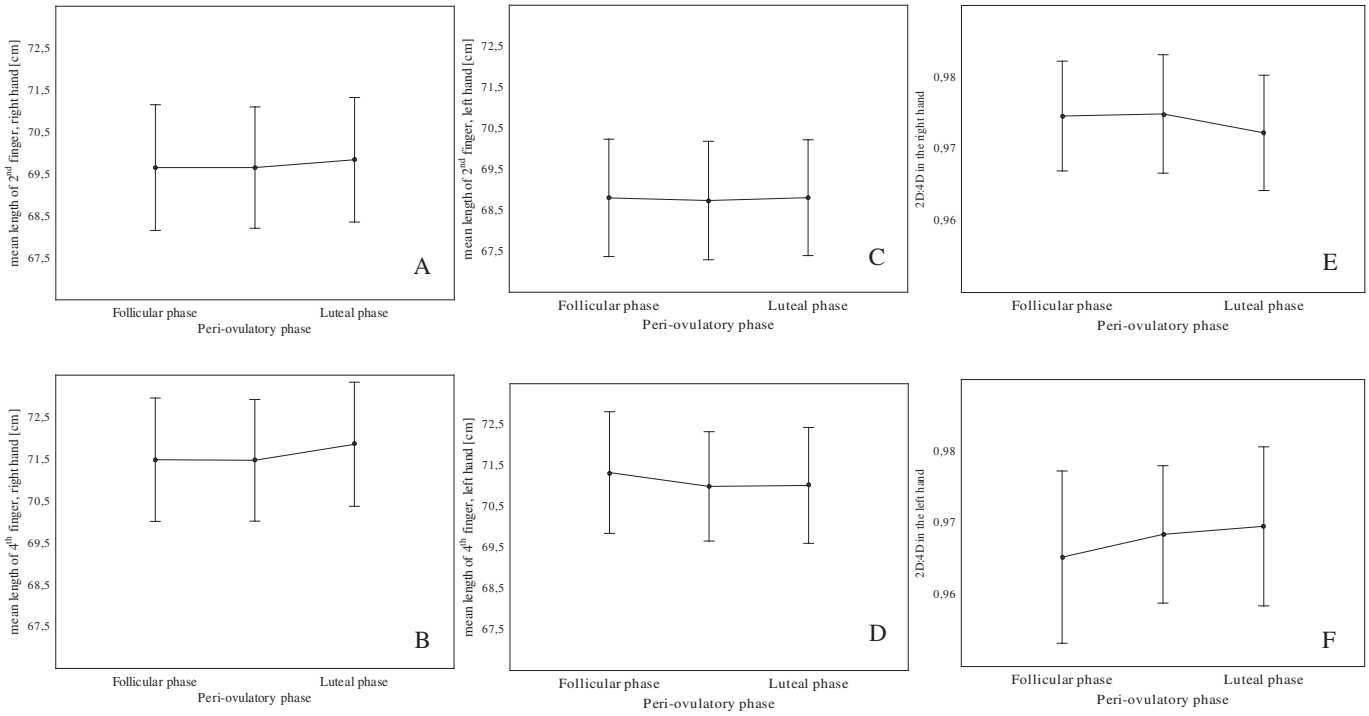

Fig. 1. Mean finger lengths in the right hand (A, B), left hand (C, D) and 2D:4D for the right (E) and left (F) hand in follicular, peri-ovulatory phase and luteal phases of the menstrual cycle.

focus on comparing the different methodological approaches in detecting possible impact of soft tissue volume on 2D:4D values.

It is worth highlighting that the main advantage of our study is the method used for ovulation detection. The range of the timing of ovulation varied among participants between the 10th and 22nd day of the cycle which suggests that defining cycle phases based on menstrual dates obtained via interview could be error-prone. Defining phases of the cycle based on LH tests for ovulation detection is much more reliable, furthermore, these tests are relatively non-expensive and easily used by study participants.

What is also worth mentioning, participants in this study were not randomly chosen but came from a rural Polish population which may raise questions as to whether these results can be generalised. Our previous hormonal studies conducted in this rural population did, however, show normal hormonal profiles in the women [18]. While these women had lower levels of ovarian hormones than urban women from the US, or Poland, they had comparable levels of hormones with women from Bolivia and Nepal, and higher levels than women from DR Congo [19]. Inter-population and inter-individual variation in ovarian hormone levels is widely observed, however, the main features of ovarian cycles (e.g. differences in levels of hormones between cycle phases) are similar among women from different populations [20,21].

In concluding, our study documented that the 2D:4D value is stable across the menstrual cycle. Small changes in finger lengths, resulting from soft tissue fluctuations on the top of the fingers, do not influence the 2D:4D value. We suggest that among naturally cycling women, the value of 2D:4D can be obtained from measurements taken during any day of the menstrual cycle. However, taking into account that we did not study randomly selected women, our participants came from a rural Polish population, these results need to be replicated in other populations.

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Ethical statement

The authors assert that all procedures contributing to this work comply with the ethical standards of The Code of Ethics of the World Medical Association (Helsinki Declaration of Helsinki), Uniform Requirements for manuscripts submitted to Biomedical Journals, and has been approved by the institutional committees (Jagiellonian University Medical College).

Contributors

All authors have made substantial contributions to the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article and finally approved the version to be submitted.

Conflict of interest statement

None declared.

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