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Experimental Study / *Deneysel Çalışma*

The Relationship Between Digit Ratio and Circadian Typology Among Medical Students

Tıp Fakültesi Öğrencilerinde El-Parmak Oranı (2D/4D) ve Sirkadyen Tipoloji Arasındaki İlişki

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Objectives: Morningness-eveningness preference stands for interindividual chronobiological differences. This study was designed to investigate if prenatal testosterone exposure is a candidate for having a causal role in sexual dimorphism observed in circadian typology.

Materials and Methods: A total of 183 students (111 females, 72 males; mean age 20.7 years) were involved in the study. Circadian typology was assessed using the Horne-Ostberg Morningness-Eveningness Questionnaire. Digit lenghts were measured with digital calipers and digit ratio (2D/4D) was calculated by dividing the length of the second digit by that of the fourth digit.

Results: Circadian typology assessment revealed 54 morning-(29.5%), 100 intermediate-(54.6%) and 29 evening-(15.8%) type students. Digit ratio of both hands in males were significantly lower than females ($p<0.001$). Digit ratio for both hands in morningness trait were significantly higher than intermediate ($p<0.001$) and eveningness ($p<0.05$ for the left and $p<0.01$ for the right hand) traits. Morning type was more common among females (35.1% vs 20.8%; $p<0.05$). Higher digit ratio was significantly associated with the morning type for both of the hands in both genders.

Conclusion: Prenatal testosterone exposure, represented by digit ratio (2D/4D) as a proxy marker, seems to have a causal role in sexual dimorphism observed in the circadian typology preferences.

Key words: Circadian typology; gender; digit ratio; fetal testosterone.

Amaç: Sabahçıl-akşamcıl tercihi bireyler arasında gözlenen belirgin kronobiyolojik farklılıkların temel dayanağıdır. Bu çalışma fetal testosteron maruziyetinin sirkadyen tiopolojiye özgü seksüel dimorfizmdeki olası nedensel rolünü araştırmak üzere tasarlandı.

Gereçler ve Yöntemler: Çalışmaya toplam 183 öğrenci (111 kadın, 72 erkek; ort. yaş 20.7) dahil edildi. Sirkadyen tip dağılımları Horne-Ostberg Sabahçıl-Akşamcıl Anketi'nin Türkçe versiyonu kullanılarak belirlendi. El parmak oranı (2D/4D) ise 0.01 mm hassasiyeti olan dijital kaliper kullanılarak elde edilen 2. ve 4. parmak uzunlukları oranlanarak hesaplandı.

Bulgular: Sirkadyen tipoloji değerlendirildiğinde, ara tipin %54.6 (n=100), sabahçıl tipin %29.5 (n=54) ve akşamcıl tipin %15.8 (n=29) oranında temsil edildiği belirlendi. 2D/4D oranı her iki elde de, erkek öğrencilerde kadın öğrencilere göre belirgin şekilde daha düşük bulundu ($p<0.001$). Benzer şekilde her iki el için saptanan 2D/4D oranları, sabahçıl öğrencilerde; ara-tipte olan ($p<0.001$) ve akşamcıl (sol elde $p<0.05$ ve sağ elde $p<0.01$) öğrencilere göre anlamlı şekilde daha yüksek bulundu. Sabahçıl tip kadınlar arasında daha yaygındı (%35.1 ile %20.8; $p<0.05$). Her iki cinste de daha yüksek 2D/4D oranı sabahçıl tipoloji ile uyumlu bulundu.

Sonuç: Fizyolojik olarak el parmak oranı (2D/4D) ile temsil edilen fetal hayatta maruz kalınan testosteron düzeyinin, sirkadyen tipolojiye özgü seksüel dimorfizmde belirleyici rolü olabileceği düşünüldü.

Anahtar sözcükler: Sirkadyen tipoloji; cinsiyet; parmak oranı; fetal testosteron.

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Being regulated by a master clock within the suprachiasmatic nucleus (SCN) of the hypothalamus,[1] circadian timing of physiological and behavioral processes is necessary for optimal functioning in mammals.

Besides daily cycle of light and darkness[2] which is the most salient external cue in the entrainment of an organism's internal circadian time to the external environmental world, internal cues such as hormonal signals secreted on a circadian basis, also target the core SCN compartment.

Experimental studies suggest that circadian period and precision, as well as the organization of daily activity bouts are dramatically affected by gonadal hormones,[3] estrogen and testosterone in the laboratory rodents.[4,5]

Considering human beings, circadian typology reflects the individual differences in human circadian biological and behavioral rhythms, as shown by the differential diurnal variation in several physiological and cognitive variables.[6-8] Morningness-Eveningness Questionnaire (MEQ) has long been used to evaluate the circadian typology[9] in terms of morning, intermediate and the evening types. According to MEQ classification, morning types (also called "larks") were defined to be more active in the earlier hours of the day preferring to go to bed early in the evening. Whereas evening types (also called "owls") were defined to wake late in the morning and to be more active in the later part of the day. Intermediate types were characterized with the patterns of behavior belonging to an intermediate area between the two extremes of this continuum.[10]

While the dominance of morningness dimension among females[11-13] was consistently reported in the past studies, there is a debate considering the role of social-cultural factors[13,14] in the existence of gender differences.[15]

It is generally assumed that the origin of gender-based differences in certain behaviors lies in neuroendocrinological events occurring during prenatal life or early postnatal life.[16] Sex hormones, mainly testosterone, seem to be associated with the development of sexually dimorphic adult behavior[17] and exert their effect early in life.[18]

It was concluded that the areas of the brain involved in language, spatial memory and motor coordination develop in a "different order, time, and rate" in females compared with males.[19,20] These differences were suggested to exist as a result of the relatively permanent, organizational effects of prenatal and early postnatal androgens, exposure to which masculinizes the nascent male nervous system.[21]

While central nervous system affects the circadian development on one hand, prenatal levels of sex steroids modulate the structure and function of the developing central nervous system[22] on the other hand. Hippocampus, hypothalamus, amygdala and prefrontal cortex have been known to be influenced by organizational effects of fetal testosterone levels.[23,24]

As certain conditions such as neurological immaturity due to preterm birth has been suggested to predispose subjects to the morningness preference,[25] studying the interaction between circadian typology and the fetal endocrine environment seems pertinent to us.

Since it is troublesome to study the direct effects of prenatal testosterone on the human fetal brain due to apparent ethical and practical reasons,[22,26] the determination of the ratio of the second to fourth finger lengths (or 2D/4D ratio)[27] has become increasingly popular. So far, digit ratio (2D/4D) is the best known physiological proxy marker of prenatal hormonal exposure which has been described to be a sexually dimorphic trait with lower mean 2D/4D in males compared to females.[28]

Based on the known interaction between gonadal hormones and sexually dimorphic adult behavior, the present study was designed to investigate the possible relation of digit ratio as a proxy marker of prenatal testosterone exposure to the circadian typology assessed among medical students.

MATERIALS AND METHODS

Subject population

Of 247 students at Baskent University Faculty of Medicine, a total of 183 students (74%) were recruited for the study upon their voluntary participation. Turkish version of MEQ questionnaire was applied via face to face interview method and digit ratio measurements were performed during the same session conducted between March 3-25, in 2008. The present study was approved by the ethics committee of Başkent University Faculty of Medicine (2007-AP-953-18.12.2007).

Digit ratio (2D/4D) measurement

Digit ratio for right and left hand was measured according to the method described by Manning et al.[27] The length of the second and fourth digits was measured on the ventral surface of the hand from the basal crease of the digit proximal to the palm to the tip of the digit with digital calipers measuring to 0.01 mm (Tresna, EC16, China). The digit ratio was calculated by dividing the length of the second digit by that of the fourth digit (2D/4D).

Morningness-Eveningness Questionnaire (MEQ Questionnaire)

MEQ developed by Horne and Östberg[9] consists of 19 items pertaining to habitual rising and bed times, preferred times of physical and mental performance and

Table 1. Digit ratio and age distribution of the subjects as a function of gender

	Male (n=72)	Female (n=111)
Age (years)	20.86±0.33	20.51±0.19
Digit ratio		
Left hand	0.96±0.04*	0.98±0.03
Right hand	0.97±0.03*	0.99±0.04

Data were shown as n(%) and mean±SEM. *p<0.001 compared to digit ratio for the both hands in the females.

subjective alertness after rising and before going to bed. MEQ yields scores ranging from 16 to 86. Higher scores (59-86) obtained from the questionnaire are suggestive of morningness whereas lower scores (16-41) are known to indicate the eveningness dimension. The psychometric properties of the Turkish version of MEQ were tested and its validity and reliability were reported to be as high as the original version.[29]

Statistical analysis

Database was transferred to SPSS (Statistical Package for Social Sciences). Statistical analysis of the data was made using SPSS 13.0 version. Chi-square ($\chi 2$) and two proportion Z test were used for the analysis of gender and circadian typology results. Student's t-test and ANOVA accompanied by post-hoc Tukey test were used to analyze gender and circadian typology scores with respect to digit ratio, respectively. Data were expressed as "mean± standard error of mean (SEM)" and percent (%) where appropriate. P<0.05 was considered statistically significant.

Table 2. Gender and age distribution of subjects as a function of circadian typology assessed by MEQ

	Circadian typology		
	Morning type (n=54)	Intermediate type (n=100)	Evening type (n=29)
Age (years)	20.62±0.29	20.67±0.26	20.62±0.17
Gender			
Male (n=72)	15 (20.8%)*	39 (54.2%)	18 (25.0%)*
Female (n=111)	39 (35.1%)	61 (55.0%)	11 (9.9%)

Data were shown as n (%) and mean±SEM. *p<0.05 compared to circaidan scores of females.

RESULTS

A total of 183 students (mean age 20.7±0.2 years) from a population of 247 (74%) were enrolled in the study. The gender distribution of our participants (females: 60.7%; males: 39.3%) was appropriate with respect to the general population (females: 59%; males: 41%). All of our students were from families of high socioeconomic status. There was no statistical difference between males and females in terms of age distribution (20.9±0.3 vs 20.5±0.2, p>0.05) (Table 1).

Digit ratio (2D/4D) of both hands was determined to be significantly lower in male students (0.96±0.04 for the left and 0.97± 0.03 for the right hand) when compared to female students (0.98±0.03; for the left and 0.99±0.04 for the right hand; p<0.001 for each) (Table 1).

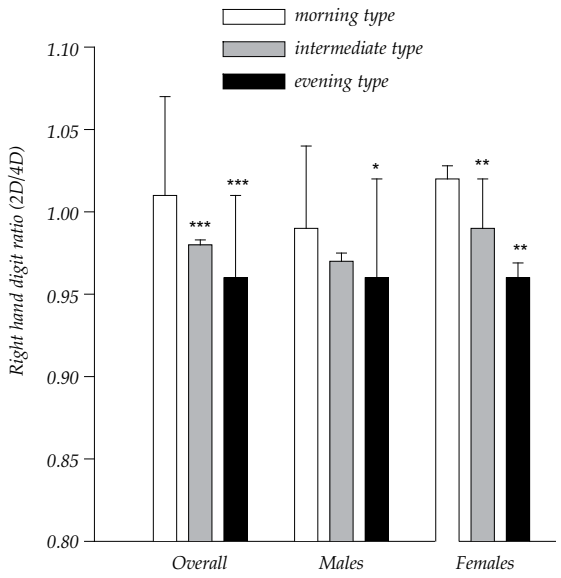

*Fig. 1. Digit ratio values for the right hand with respect to circadian typology preferences among overall (n=183), male (n=72) and female (n=111) students. *p<0.05, **p<0.01 and ***p<0.001 compared to digit ratio for the right hand in the morning types.*

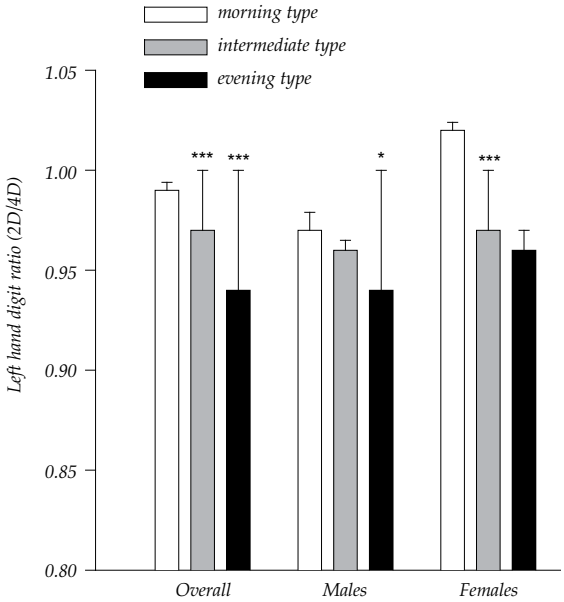

*Fig. 2. Digit ratio values for the left hand with respect to circadian typology preferences among overall (n=183), male (n=72) and female (n=111) students. *p<0.05, **p<0.01 and **p<0.001 compared to morning type digit ratio for the left hand in the morning types.*

When circadian typology is considered, there were 54 morning- (29.5%), 100 intermediate- (54.6%) and 29 evening-type (15.8%) students based on their MEQ scores. (Table 2). There was a significant gender difference in the circadian typology (Table 2). Morning type was defined by 20.8% of males but 35.1% of females. Intermediate type was defined by 54.2% of males and 55.0% of females. Evening type was defined by 25.0% of males and only 9.9% of females ($p<0.05$) (Table 2). Age distribution of morning- (20.6±0.3), intermediate- (20.7±0.3) and evening-type (20.6±0.4) subjects was similar ($p>0.05$; Table 2).

In general, higher digit ratios were significantly associated with the morning type for both of the hands. So, 2D/4D for the right hand was markedly higher in morning types (1.01±0.06) when compared to intermediate (0.98±0.003; $p<0.001$) and evening (0.96±0.05; $p<0.001$) types (Fig. 1). Similarly 2D/4D for the left hand was also markedly higher in morning types (0.99±0.004) when compared to intermediate (0.97±0.03; $p<0.001$) and evening (0.94±0.06; $p<0.001$) types (Fig. 2).

When digit ratio of males and females was evaluated separately in terms of circadian typology, higher 2D/4D ratios were determined to be associated significantly with morning types in both genders. For the right hand, morning-type males (0.99±0.05) had significantly higher 2D/4D when compared to evening-type males (0.96±0.06; $p<0.05$) (Fig. 1). Similarly, right hand 2D/4D in the morning-type females (1.02±0.008) was significantly higher than intermediate- (0.99±0.03; $p<0.01$ and evening-type (0.96±0.009; $p<0.01$) females (Fig. 1). For the left hand, morning-type males (0.97±0.009) had significantly higher 2D/4D when compared to evening-type males (0.94±0.06; $p<0.05$) (Fig. 2). Similarly, left hand 2D/4D in the morning-type females (1.02±0.004) was significantly higher than intermediate- (0.97±0.03; $p<0.001$ and evening-type (0.96±0.01; $p<0.01$) females (Fig. 2).

DISCUSSION

Morningness and eveningness are the well-known extremes of a spectrum referring to the classification of individuals with respect to their preferred time for certain physiological and behavioral activities.[8]

Distinct functions of neurons within separate SCN sub-regions in the overall organization of the circadian clock and sex differences in the androgen receptor density together were stated to point to a convergence of endocrine and neural input to SCN elsewhere in the literature.[30] Therefore, the likelihood of this arrangement provides the male–female differences in integration of environmental and internal cues modulating circadian rhythmicity.[31]

In agreement with the previously reported negative correlation between the digit ratio (2D/4D) and the prenatal testosterone exposure in the fetal life,[27,28] male students were determined to have lower digit ratios than females for both hands in the present study. In fact, a significant relationship between the behavioral traits and 2D/4D has been reported more frequently for the right hand which was considered to be affected by androgenization more than the left one.[27,28] However, we have found significant relationships between circadian typology and the digit ratio for both hands in our study.

As far as the gender differences are concerned, our results are consistent with the past studies suggesting an inclination towards morning-disposition among women.[7,32] Futhermore, providing an explanation for the factors underlying the gender differences in the circadian typology, morning type predominance was observed not only among females but also in males who have higher digit ratios than their conspecifics in the present study.

Modulatory effects of gonadal hormones on circadian behavior and physiology have been reported to occur via androgenic hormones which act through SCN androgenic receptors allowing males and females to display similar temporal behavior patterns.[30] It was stated that direct SCN projections to neuroendocrine cells were associated with the generation of steroid-induced LH surges.[33] Therefore, sex differences in SCN androgen receptors may reflect male-female differences in GnRH regulation.[34]

University students were reported in the literature to be biased towards the evening dimension that accounts for 20-30% with respect to the studied population.[35] In contrast, the circadian inclination of our students was towards the morningness dimension (29.5%). Moreover, the morningness dimension identified by our students was also higher than the percentage reported (15%) in the past studies conducted with people aged from 20 to 50.[36,37]

Since morningness orientation was related to higher conscientiousness, self-esteem, and focus of control[38] while eveningness has been related to depressive tendencies,[39] low academic performances and difficulties in social adaptation,[40] striking difference concerning circadian inclination among our medical students from high status families may indicate the role of socio-cultural and ethnic factors besides the special needs of medical education in the configuration of circadian typology. Accordingly, none of our medical students in the clinical period was from eveningness trait. Since eveningness trait seems to contradict the success and safety concerning medicine, medical education itself may induce the likelihood of morningness trait among its members. In fact, work schedules were known to act as a powerful "zeitgeber"[7] and university students seem to be more likely to adapt their demands to their biological preference.[41]

According to our results, as an index of low fetal testosterone exposure, high digit ratio might be concluded to predispose the subjects to morningness preference in both genders. Therefore, digit ratio may be a useful index in the prediction of gonadal effects underlying different chronotypes of human circadian clock. This prediction might be advantageous for evening-type students in terms of bracing for the future; since greater behavioral troubles, low academic performances and difficulties in social adaptation associated with their circadian preference compose a significant disadvantage for the medical education.

The main limitation of our study is that it involves results for the very specific group of medical students representing high socioeconomic level population that can not be generalized to the whole medical students. Another limitation is the lack of a clear cut explanation concerning higher prevalence of morningness trait among our students compared to other universities which in part may be caused by the limited number of reference studies conducted in our country.

In conclusion, gender differences in circadian typology among people seem to be associated with in utero organizing effects of prenatal testosterone on the fetal brain as far as our medical students are concerned. Hence, represented by digit ratio (2D/4D) as a proxy marker, prenatal testosterone exposure may have a causal role in sexual dimorphism observed in the circadian typology preferences.

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