Comparison of the Ratio of the Lenght of the Second and Fourth Digits in Subgroups of Fertile and Infertile Cases

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Purpose: To identify any relationship between known reasons of male infertility and 2D:4D ratio.

Materials and Methods: A total of 371 males were included in the study. The cases were grouped into 6 groups including sperm count < 5 million/mL, sperm count ≥ 5 million/mL, Klinefelter Syndrome, hypogonadotropic hypogonadism, vasal agenesis and control. Groups were compared with each other in terms of 2D:4D ratios and groups with a 2D:4D ratios below 1 and equal/above 1 were compared.

Results: The greatest ratios were in the vasal agenesis and hypogonadotropic hypogonadism groups and analysis of the data with logistic regression analysis showed that there was a significant difference in terms of 2D:4D ratios for these groups when comparing with control group. The other groups showed no statistically significant differences.

Conclusion: The results of the present study showed some significant difference between 2D:4D ratios for the subgroups of the fertile and infertile cases. Although, 2D:4D ratio is not an unaccompanied parameter to reveal causes of male infertility, it can be associated with some situations that are related with male infertility.

Keywords: 2D:4D; digit lenght ratio; fluctuation asymmetry; hox gene; infertility.

INTRODUCTION

In vertebrates Homeobox (Hox) gene family is necessary for development of limbs and genitalia[1,2]. The members of Hox gene family, Hoxa and Hoxd, are needed for differentiation of the genital bud and growth and formation of the digits[3].

It has been suggested that the relationship between those 2 distinct body parts is not solely the common genetic control during their developmental stages, but also other factors may be effective. Among those factors, prenatal sex steroids are the most commonly studied. Differences in prenatal hormone levels in female and male fetuses cause their genital developments to progress in different directions after a certain step. In men second digit is typically shorter than 4th digit whereas second digit in women is generally equal to or longer than 4th digit. Hence, length of the 2nd digit to that of 4th digit (2D:4D ratio) is less than 1 in majority of men and equal to or greater than 1 in most women[4].

We aimed in this study, by hypothesizing that 2D:4D ratio could be related to infertility, to identify any relationship between known reasons of male infertility and 2D:4D ratio.

MATERIALS and METHODS

Study Design

A non-interventional, prospective and cross sectional study was designed in Erciyes University Urology Department between April 2011 and May 2013.

Study Population

A total of 331 men who attended to our clinic with complaints of infertility and 40 men who fathered at least one child were included the study. Cases with congenital or acquired digit deformity in 2nd or 4th digit of the right or left hand were excluded.

Evaluations

Palmar surfaces of the right and left hand of the cases were transferred to digital medium with the help of a scanner (Hewlett Packard® Scanjet G3110). Lengths of 2nd and 4th digit were measured with a digital caliper with a sensitivity of 0.01 mm (Mitutoyo®). To measure the length of 2nd digit, the distance between the midpoint of the proximal line separating the digit stem from palm and the tip of the index digit was used. To measure the length of 4th digit, the distance between the midpoint of the proximal line separating the digit stem from palm and the tip of the fourth digit was used. The distances were measured in millimeters and recorded. Next, the ratio of 2nd digit to 4th digit (2D:4D) was calculated and recorded for both hands in each case.

At a later stage of the study, a semen analysis was performed in cases other than those in fertile control group at least one time and preferably 2 times and the analysis was evaluated according to the World Health Organization (WHO) 2010 criteria[5].

Outcomes

With laboratory and physical examination findings, the cases were grouped into 6 groups: 46 XY-genotype cases with nonobstructive azoospermia and severe oligoastenozoospermia (sperm count less than 5 million/mL) (n = 115), cases with a sperm count above 5 million/ml (n = 90), cases with Klinefelter Syndrome (n =

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Table 1. Distribution of the groups according to 2D:4D ratio.

Group no	Groups	Right 2nd and 4th Digit Ratio		Left 2nd and 4th Digit Ratio		
		< 1	≥ 1	< 1	≥ 1	
		n, (%)	n, (%)	n, (%)	n, (%)	Total
1	Sperm count < 5 million/mL	104 (90.4)	11 (9.6)	97 (84.3)	18 (15.7)	115
2	Sperm count ≥ 5 million/mL	77 (85.6)	13 (14.4)	73 (81.1)	17 (18.9)	90
3	Klinefelter Syndrome	60 (92.3).	5 (7.7)	58 (89.2)	7 (10.8)	65
4	Hypogonadotropic Hypogonadism	22 (81.5)	5 (18.5)	20 (74.1)	7 (25.9)	27
5	Vasal Agenesis	24 (70.6)	10 (29.4)	25 (73.5)	9 (26.5)	34
6	Control Group	36 (90.0)	4 (10.0)	38 (95.0)	2 (5.0)	40
	Total	323 (87.1)	48 (12.9)	38 (95.0)	60 (16.2)	371

65), cases with hypogonadotropic hypogonadism (n = 27), cases with vasal agenesis (n = 34), and the control group with children (n = 40).
Groups were compared with each other in terms of 2D:4D ratios and groups with a 2D:4D ratios below 1 and equal/above 1 were compared.

Ethics

Informed consent was obtained from all cases. The study was approved by the ethics committee.
Statistical Analysis:
All statistical analyses were performed using SPSS 20.0 for Windows® statistical software package (Chicago,USA). Categorical variables were compared using logistic regression analysis. A p value less than .05 was considered statistically significant.

RESULTS

Assessment of cases with a 2D:4D ratio in right hand equal to or greater than 1 with other cases in their group revealed that this condition is present in 11 of 115 cases (9.6%) with Sperm count < 5 million/mL (Group 1), 13 of 90 cases (14.4%) with a sperm count above 5 million/mL (Group 2), 5 of 65 cases (7.7%) with Klinefelter Syndrome (Group 3), 5 of 27 cases (18.5%) with hypogonadotropic hypogonadism (Group 4), 10 of 34 cases (29.4%) with vasal agenesis (Group 5), and 4 of 40 cases (10%) in the control group (Group 6). Distribution of the groups for right hand was shown in **Table 1**.
The greatest ratios were in the vasal agenesis and hypogonadotropic hypogonadism groups and analysis of data with logistic regression analysis showed that there was a significant difference between control group and vasal agenesis group in terms of 2D:4D ratios (*P* = .041)(**Table 2**).
2D:4D ratios were also assessed in left hand. Number of cases with a 2D:4D ratio equal to or above 1 were 18 (15.7%) in group 1, 17 (18.9%) in group 2, 7 (10.8%) in group 3, 7 (25.9%) in group 4, 9 (26.5%) in group 5, 2 (5%) in group 6 (**Table 1**). Like the right hand, higher ratios were detected in hypogonadotropic hypogonadism and vasal agenesis groups. Both groups showed statistically significant differences when compared with the control group. *P* values were .025 and .019 respectively (**Table 2**).

DISCUSSION

This clinical study compared infertile case groups with fertile control group separately in right and left hand in terms of that the digit ratio is below 1, or equal to or greater 1. The percentage of cases with a 2D:4D ratio equal to or greater than 1 was specially higher among vasal agenesis and hypogonadotropic hypogonadism groups and some statistically significant differences were detected.
The discovery that Hox genes are necessary for devel-

Table 2. Comparison of the subgroups according to 2D:4D ratio with logistic regression analysis.

	Right hand	*p*	Left hand	*p*
	OR (95%CI)		OR (95%CI)	
Control group	1.00	-	1.00	-
Group 1	0.95 (0.29-3.18)	.936	3.53 (0.78-15.93)	.102
Group 2	1.52 (0.46-4.99)	.490	4.43 (0.97-20.16)	.055
Group 3	0.75 (0.19-2.98)	.682	2.29 (0.45-11.63)	.316
Group 4	2.05 (0.50-8.44)	.323	6.65 (1.26-35.05)	.025
Group 5	3.75 (1.05-13.35)	.041	6.84 (1.36-34.33)	.019

opment of genital bud and growth of the digits followed by demonstration of Hox gene expression products in spermatozoa after meiotic division has led to the idea that Hox genes and hence digit characteristics may be related with fertility[6]. Manning et al. studied the relationship between 2D:4D ratio and sperm count. They compared 12 males with germ cell failure with 46 males with normal semen parameters in terms of right and left hand 2D:4D ratios. They reported a significant difference in right hand ratios and an insignificant difference in left hand ratios[7]. The number of cases and subgroups in our study was much greater than this study. In this way, some comments can be done about which male infertility reason is more relevant with digit ratios.

The studies supporting the relationship between fertility and digit ratio are also not scarce. Wood et al. conducted a study with 44 cases to explore this relationship. Among their study population, 16 cases had nonobstructive azoospermia, 4 had bilateral vasal agenesis, and 24 previously fertile vasectomized cases had azoospermia. The authors investigated the relationship between the success of surgical sperm extraction and 2D:4D ratios in these groups. Furthermore, they also compared the rates of having children and clinical pregnancy rates with 2D:4D ratios in cases with a successful sperm extraction procedure. This study found that 2D:4D ratios were lower in previously fertile vasectomized patients compared with nonobstructive azoospermic cases. Cases with nonobstructive azoospermia who had a successful sperm extraction procedure had a lower 2D:4D ratio in right hand than those with an unsuccessful procedure. None of the cases with a successful sperm extraction procedure had a left hand 2D:4D ratio greater than 1. The pregnancy success in cases with successful surgical extraction was not affected by 2D:4D ratio[8]. To our opinion, the most important point in this study that can be criticized is that the cases with bilateral vasal agenesis were not assessed as a separate group but rather included in a larger group, largely because their number was low. That is because the cases were divided into two large groups as congenital and acquired azoospermic cases and the cases with bilateral vasal agenesis were assessed as a whole with nonobstructive azoospermia and compared with vasectomized cases..

The pathology in cases with vasal agenesis is the absence of ductus deferens. Most of the testicular functions are intact in these cases and the chance of finding sperms surgically is high[9]. Therefore, the presence of this small group that has a high rate of sperm extraction in that study with an already small sample group may highly influence the results. In addition, the criticism of small sample size made for similar studies also applies to that study. If the number of cases in this group was higher and these cases were evaluated as a separate group, the results would be clearer.

Examining the situation from a different perspective may be more clarifying to assess the relationship between digit ratios and fertility. That perspective is whether there is a relationship between digit ratios and sex hormones. The evidence from majority of these studies have suggested that differences in androgen and estrogen levels during development are indirectly related with 2D:4D ratio[10]. It is currently unknown how sex steroids influence the developmental stages in digit development and how prenatal androgen and estrogens play a role in the gender difference between digit ratios. Experimental animal studies have been conducted to partly elucidate this relationship. The study that has drawn the most attention was that conducted on mice. According to this animal study, 2D:4D ratio is determined by a balance of prenatal testosterone and prenatal estrogen signalization within a short period of fetal life. An important result of that study was that external hormones and receptor antagonists applied postnatally did not alter 2D:4D ratio at the postnatal development period, but it did affect anogenital distance. Therefore, it has been suggested that 2D:4D ratio is determined during embryonic period and remains constant for a life time[11]. Percent of cases with hypogonadotropic hypogonadism that had 2D:4D ratio equal to or greater than 1 was high in our study and a significant difference was detected for left hand when the cases with hypogonadotropic hypogonadism were compared with fertile males. This may be related to a hormonal irregularity at their intrauterine micro medium during prenatal period.

Like digit length ratio, fluctuation asymmetry is also considered to be related with many physiological and pathological conditions. Bilateral paired morphologic features show excellent symmetry and stability during developmental process. However, random deviations from symmetry may take place during development and this phenomenon is called the fluctuation asymmetry, which is assessed by measuring the differences between morphologic features showing symmetry (ears, index digits, wrists, ankles etc.) with different methods[12]. A study by Firman et al. investigated whether semen quality is reliably reflected by digit length ratios and the fluctuation asymmetry. Fifty cases aged 18-35 years were included. Cases underwent semen analysis, fluctuation asymmetry measurements, and 2nd-4th digit length measurements to calculate 2D:4D ratio. The results showed a significant relationship between body fluctuation asymmetry and total sperm count, sperm motility, and sperm head length. However, no significant relationship was evident between 2D:4D ratio and semen parameters for both hands[13]. The fluctuation asymmetry and differences in digit length ratios which may be related with physiologic and/or pathologic conditions may actually be morphologic features partly reflecting the functionality or disorder of the same common developmental mechanisms. In this context, fluctuation asymmetry and 2D:4D ratio could be a predictor for vasal agenesis which can be regarded as a developmental mechanism disorder.

Our control group was composed of men who fathered at least one child and they were assumed as fertile cases. No semen analysis was performed for this group because of the costs and the ethical reasons. However, it is possible for a father having an abnormal spermiogram. Creating a control group from individuals with normal semen analysis could give more clear results. So, this was a limitation of our study.

CONCLUSIONS

The results of the present study showed some significant difference between 2D:4D ratios for the subgroups of the fertile and infertile cases. Nevertheless, 2D:4D ratio is not an unaccompanied parameter to reveal all causes of male infertility. Because the number of factors that have a role in this condition is probably more than known. However, 2D:4D ratio may be a good predictor for some rare subgroups of infertile men like

vassal agenesis and hypogonadotropic hypogonadism. Future large scale studies may identify more specific relationships among these situations and 2D:4D ratio.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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