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Digit Ratio is Associated with Colorectal Cancer

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Abstract

Background: Prenatal testosterone and estrogen exposure may influence disease susceptibility. Digit ratio (2D:4D) is a putative marker for prenatal hormone exposure and sensitivity, as well as the action of genes related to malignancies. Therefore, 2D:4D could act as a marker for cancer predisposition.

Aims: Investigate the possible correlation between R2D:4D, L2D:4D and right minus left. 2D:4D (Δr-l) and colorectal cancer (CRC) in men and women and assess correlations with tumor staging and histological diagnosis.

Methods: Digital images of the right and left hand palms of patients diagnosed with CRC (n=139) and age and sex-matched controls (n=139) were used to measure the 2nd and 4th fingers. Means for 2D:4D were compared. Data were analyzed by intraclass correlation coefficient, Student's t-test, Kendall's tau b and Spearman's rho (α=0.05).

Results: CRC group presented significantly higher right and left 2D:4D (p=0.00001 and p=0.00005, respectively) in comparison to healthy controls. R2D:4D was negatively correlated to tumor differentiation (p=0.02) while L2D:4D was positively correlated to tumor size (p=0.03).

Conclusions: Prenatal estrogen and testosterone seem to play a role on the malignant transformation and progression of colorectal cancer. The findings suggest that 2D:4D could add to the list of etiological factors and be a putative marker for the screening of patients' susceptibility to develop colorectal cancer.

Keywords: Digit ratio; 2D:4D; Colorectal cancer

Introduction

The ratio between the 2nd and 4th digits, also called 2D:4D is a putative marker for the balance between prenatal exposure and sensitivity to testosterone and estrogen [1,2] and for the action of genes involved in the regulation of body development [3-7] (Table 1). Prenatal Testosterone (PT) is thought to be inversely correlated to 2D:4D in the right and left hands, as well as in the difference between the finger-length ratios of both hands (Δr-l) [8,9]. Evidence shows that 2D:4D may be predictive of susceptibility to cancers, particularly those that show sex differences in their occurrence, progression, and prognosis [3]. Moreover, the genes correlated to 2D:4D determination seem to be closely related to the incidence and progression of different types of cancer, including colorectal tumors.

Prenatal testosterone				Colleteral cancer
				Interaction
Testosterone			-	Incidence
Estrogen			-	Progression
Fgfr2	+	Ø	+	Differentiation
Sox9	+	Ø	+	Primary tumors
Col4a2	+	Ø	+	Weak evidence
Col12a1	+	Ø	-	Microsattelite instability
Bmp6	-	Ø	-	Progression
Smad3	-	Ø	-	Progression
Wnt5a	-	Ø	-	Metastasis
Runx2	-	Ø	-	Incidence
Mmp9	-	Ø	+	Incidence
Col6a2	Ø	+	+	Incidence
Fgf3	Ø	-	+	Progression
Msx1	Ø	-	+	Metastasis
Igfbp5	-	-	+	Incidence

Table 1: Correlation between gene expression, prenatal testosterone and colorectal cancer in men and women. + - Positive correlation; - - Negative correlation; Ø – No known correlation; - Males; - Females; CRC Colorectal cancer.

Colorectal cancer (CRC) is the second most prevalent cancer in men and the third in women, with a reported incidence of 56.06/100,000 (66.14/100,000 for males and 48.66/100,000 for females)

and 143,460 new cases expected to be diagnosed in 2012 [10-12]. The majority of cases (94%) correspond to sporadic adenocarcinomas. Approximately 5% are directly correlated to genetic disorders known as Familial Adenomatous Polyposis (FAP) and Hereditary Nonpolyposis Colorectal Cancer (HNPCC) also known as Lynch syndrome [12,13]. The main risk factors for sporadic CRC are diet and obesity, heavy tobacco and alcohol consumption as well as sedentary lifestyle and familial history of colorectal malignancy [14].

This study investigated possible correlations between colorectal cancer and 2D:4D in men and women in retrospective fashion. Given the diagnosis of CRC is often delayed, we hypothesized that cases would present a higher 2D:4D or R-L 2D:4D - and subsequently higher exposure and sensitivity to prenatal estrogen and lower exposure to testosterone compared to age-matched controls - and that 2D:4D is positively correlated to the clinical staging in colorectal cancer. As colorectal cancer is more frequent in males than females [10] and estrogen may be chemopreventive for colorectal cancer [14-16], we predicted that low 2D:4D (or low R-L 2D:4D) would be associated to tumor differentiation in colorectal cancer risk.

Methods

This research is in accordance with the principles laid down in the Declaration of Helsinki, amend of 2008. Subjects aged 40-80 years were invited for the study when attending follow-up of cancer treatment or chemotherapy sessions and responded to a questionnaire regarding tobacco and alcohol consumption, medical and familial history. Controls were healthy subjects, age and sex-matched to the patients diagnosed with colorectal cancer. Physical activity status was not assessed. Volunteers with index or ring finger fractures, hormonal disorders and/or BMI ≥ 35 were excluded from the study. Participants then had their right and left hand palms photographed according to the methodology proposed by Hopp and Jorge [17].

Image analysis was performed using Adobe Photoshop CS5® (Adobe Systems, USA) measuring tool (100% zoom). Index and ring fingers were measured twice from the middle point of the most proximal crease up to the tip. Index finger length was divided by ring finger length, originating the 2D:4D ratio. The analyzed ratio was the mean of the two measurements performed. The left-hand 2D:4D was subtracted from the right-hand 2D:4D, originating the right minus left 2D:4D (Δr-l).

Individuals were then assigned to their respective groups, subdivided by gender: (a) CON group – patients without present or past malignant lesions; (b) CRC group – patients diagnosed with sporadic colorectal cancer by digital examination, colonoscopy and histological examination. This group was subdivided into Colon cancer and Rectum cancer subgroups. Histological sub typing (well, moderately and poorly differentiated) and TNM staging were also recorded for analysis. Data were analyzed by intraclass correlation coefficient and Student's t-test for finger measurements and group comparisons, Kendall's tau b for correlations with tumor staging and Spearman's rho for histological correlations using SPSS®19 (α=0.05).

Results

Characteristics of the population and correlational data are described in Table 2. Overall, 278 individuals were selected for this study (140 males, 70 patients and 70 age-matched controls; 138

females, 69 patients and 69 age-matched controls). Alcohol consumption and familial history of malignancy were not frequent. Mean cigarette and alcohol consumption were lower on the CRC group. Hormone therapy was not reported. Colon cancers comprised 81 cases (35 males, 46 females, age range 35–79 years) and rectal cancers comprised 58 cases (32 males, 26 females, age range 32–79 years). The majority of colon cancers were moderately differentiated adenocarcinomas (74%) and the same was observed for rectal cancer (76%).

	Colorectal Cancer (CRC)	Controls (CON)	p
Age (Mean, SD)	58.5 (11.9)	57.9 (11.9)	
Tobacco consumption	9%	11%	
Alcohol Intake	30%	42%	
Right 2D:4D (Mean, SD)	0.971 (0.044)	0.949 (0.038)	0.00001
Left 2D:4D (Mean, SD)	0.966 (0.041)	0.947 (0.039)	0.00005
Δr-l 2D:4D (Mean, SD)	0.004 (0.047)	0.002 (0.044)	0.34

Table 2: Characteristics of the patients by colorectal cancer status. SD – Standard deviation.

Intraclass correlation coefficient was 0.792 (p=0.00; F=4.79) between measurements for the right hand and 0.968 (p=0.000 F=30.75) for the left hand, indicating differences in

2D:4D between individuals were greater than measurement error. The mean 2D:4D from the first and second measurements was used. CRC group (n=139; 70 males) had mean R2D:4D of 0.971 ± 0.044 and mean L2D:4D of 0.9663 ± 0.0412; CON group (n=139; 70 males) had mean R2D:4D of 0.9497 ± 0.0383 and L2D:4D of 0.9471 ± 0.0394. Mean Δr-l

2D:4D was 0.004 ± 0.047 for the DIS group and 0.002 ± 0.044 for the CON group. Right and left hand digit ratios differed significantly between cancer patients and controls (p=0.00001 and 0.00005, respectively) in men and women (p=0.015 and p=0.0001 for men; p=0.018 and 0.0004 for women for right and left hands respectively) but Δr-l did not (p=0.34; p=0.46 and 0.32 for males and females respectively). Meanwhile, R2D:4D, L2D:4D and Δr-l were not significantly different between males and females with colon or rectal cancer. Rectal cancer showed lower mean L2D:4D than colon cancer (0.961 versus

0.975, p=0.04), while R2D:4D and Δr-l had no significant differences. L2D:4D was positively correlated to tumor size (T) in colon cancers (t= 0.195; p=0.027) but not in rectal cancers (t=0.134, p=0.146). Lymph node involvement (N) was significantly correlated to R2D:4D in rectal cancers (t=0.268, p=0.023) but not in colon cancers (t=-0.013, p=0.44). Distant metastases (M) and clinical staging (CS) did not correlate significantly to R2D:4D, L2D:4D or Δr-l. for colon or rectal cancers. Tumor differentiation did not present significant differences between R2D:4D, L2D:4D or Δr-l for colon cancer. However, R2D:4D was significantly negatively correlated to tumor differentiation in rectal tumors (r^2=-0.37, p=0.19), while L2D:4D and Δr-l were not (Table 3).

	Colon cancer (t, p)					Rectal cancer (t, p)				
	T	N	M	CS	TD (r², p)	T	N	M	CS	TD (r², p)
Right 2D:4D	.082 (0.209)	-0.13 (0.45)	0.05 (0.32)	0.003 (0.49)	0.07 (0.31)	0,10 (0.21)	0.26 (0.02)	0.11 (0.21)	0,20 (0.06)	-0.37 (0.02)
Left 2D:4D	0.19 (0.03)	-0.08 (0.21)	-0.12 (0.12)	-0.09 (0.15)	-0.05 (0.38)	0.13 (0.14)	0.15 (0.12)	0.17 (0.10)	0.17 (0.09)	-0.09 (0.32)
Δr-l 2D:4D	-0.06 (0.26)	0,08 (0.18)	0,14 (0.88)	0,08(0.20)	0.14 (0.17)	-0.03 (0.41)	0.06 (0.33)	-0.10 (0.24)	0,004 (0.48)	-0.28 (0.06)

Table 3: Correlations between 2D:4D, TNM, clinical staging and histological type in colon and rectal cancers. T: Tumor size; N: Local lymph node involvement; M: Distant Metastases; CS: Clinical Staging; TD: Tumor Differentiation; t: Kendall's Tau b; r²: Spearman's Rho; Δr-l: Right minus left 2D:4D

Discussion

Sex steroids seem to play an important role on the incidence and progression of colorectal cancer. However, there seems to be a U-shaped effect on the carcinogenesis and prognosis of this malignancy, where low testosterone seems to play an important role on the malignant transformation and differentiation [18] and estrogen seems to be negatively correlated to tumor progression [19]. The genetic influence in colorectal cancer seems to be especially correlated to incidence, progression and metastasis, influenced by genes that are also correlated to digit ratio establishment (Table 1) [7]. It is interesting to note that those genes can be positively correlated to prenatal testosterone (hence negatively correlated to prenatal estrogen) or vice-versa.

The results are consistent with the predictions and point towards the influence of high prenatal estrogen on the development of colorectal cancer, while establishing a positive correlation between testosterone and tumor differentiation. The high R2D:4D and L2D:4D of cases compared to controls can be correlated to the tumor symptoms that led the patients to seek treatment. Recent studies have identified strong correlations between PT and right, left 2D:4D and Δr-l 2D:4D [8,9] confirming previous inferences of correlations between prenatal hormones and 2D:4D [1].

It is important to note that screening for colorectal cancer, although advised to start at age 50, is often overlooked [20], which can explain the positive correlation between high L2D:4D and tumor size found in this study. Higher influence of prenatal estrogen could be associated to lower sensitivity to circulating hormone later in life, thus explaining the correlation between high 2D:4D and tumor incidence. An interesting finding is the absence of correlation between Δr-l 2D:4D and all colorectal cancer features, pointing to a possible interaction between prenatal testosterone and estrogen in a different way than those previously reported [8,21],where the former would play a role on malignant transformation while the other would be key for progression, as evidenced by the higher R2D:4D for moderately-differentiated rectal tumors in comparison to well-differentiated

In addition to contributing for progression, prenatal estrogen – as evidenced by high R2D:4D and L2D:4D – can be linked to heavy tobacco and alcohol consumption, two important risk factors for colorectal cancer, as tobacco consumption seems to be positively correlated to testosterone and heavy alcohol consumption could be positively correlated to estrogen [22]. Digit ratio could also be connected to physical fitness [23], and one can infer its relationship to obesity, another important risk factor for colorectal cancer. These main etiological factors were considered, and it is important to note

that there are not large differences in ethnicity regarding colorectal cancer incidence, which would make it difficult to control for in an admixed population as the Brazilian. As this is a retrospective study, it is possible that cases had been diagnosed already in advanced stage, which is common for colorectal cancer, since these tumors present unspecific signs or symptoms until they have progressed too far [24] To allow for standardization, only non-syndromic patients were selected for the study, as there is much greater genetic influence in patients with FAP or HNPCC and this could place a shadow over the influence of 2D:4D in tumor prevalence.

Prenatal estrogen was correlated to colorectal cancer in males and females, as evidenced by high R2D:4D and L2D:4D, the latter particularly in rectal cancer, which can indicate an even higher influence of estrogen in this cancer subtype. This study provides evidence for correlations between colorectal cancer and 2D:4D, suggested as a putative marker for the development of these tumors, adding to the list of cancers correlated to 2D:4D [8,17,21,25-27]. It also points to the necessity to investigate relations between genetic and hormonal factors represented by 2D:4D that could affect the sensitivity of cellular membrane receptors to circulating hormones in adult life.

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