RESEARCH ARTICLE

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Main Risk Factors Association with Proto-Oncogene Mutations in Colorectal Cancer

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Abstract

Objective: Although several factors have been shown to have etiological roles in colorectal cancer, few investigations have addressed how and to what extent these factors affect the genetics and pathology of the disease. Precise relationships with specific genetic mutations that could alter signaling pathways involved in colorectal cancer remain unknown. We therefore aimed to investigate possible links between lifestyle, dietary habits, and socioeconomic factors and specific mutations that are common in colorectal cancers. **Methods:** Data were retrieved from a baseline survey of lifestyle factors, dietary behavior, and SES, as well as anthropometric evaluations during a physical examination, for 100 confirmed primary sporadic colorectal cancer patients from Northwest Iran. **Results:** High socioeconomic status was significantly associated with higher likelihood of a *KRAS* gene mutation ($P < 0.05$) (odds ratio: 3.01; 95% CI: 0.69–13.02). Consuming carbohydrates and alcohol, working less, and having a sedentary lifestyle also increased the odds of having a *KRAS* mutation. **Conclusion:** Although research has not yet described the exact relationships among genetic mutations with different known risk factors in colorectal cancer, examples of the latter may have an impact on *KRAS* gene mutations.

Keywords: Colorectal cancer- risk factors- *KRAS*- regression

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Introduction

Several factors have known etiological roles in colorectal cancer (CRC), including a sedentary lifestyle, obesity, specific hormone treatments, smoking, alcohol use, and taking non-steroidal anti-inflammatory (NSAIDs) drugs (van Engeland et al., 2003; Brim et al., 2008; Heine-Broring et al., 2015). CRC causes almost 700,000 deaths annually (Noll and Ferrante, 2016), and as countries become more affluent, the increasing tendency of the population to adopt Western diets and lifestyles increases the risk of developing CRC (Malekzadeh et al., 2009).

According to last updated publication of WCRF/AICR regarding the association between diet, nutritional, and physical activity and the incidence of CRC, these risk factors classified as "convincing" and "probable" known risks that affect the incidence of colorectal cancer according to evidences (Research., 2017), but the precise relationship between these factors and specific genetic mutations that could alter signaling pathways is unknown (Doria-Rose et al., 2016). Recent studies have shown that CRC happen through different molecular pathways with distinct genetic alterations linked to demographic and environmental factors as well as family history (Kin et al., 2013). However, few studies have examined the relationship among genetic mutations and nutritional factors including processed meat, tea, coffee, tobacco, and alcohol consumption with lifestyle in CRCs. (Cummings and Bingham, 1998; Kratz et al., 2007; Kamal et al., 2012). In addition, there is evidence that a sedentary lifestyle and prolonged immobility increases the risk of CRC (Samowitz et al., 2006; Slattery et al., 2007). However, the molecular mechanisms underlying these relationships with the development of CRC are not completely understood.

Socioeconomic status (SES) can be extremely related to habits, behaviors, and decisions in life. The results of previous studies of SES and the risk of developing CRC have shown that higher socioeconomic classes have higher CRC risks (Wu et al., 2006; Aarts et al., 2010; Boyle et al., 2014).

Activation of Kirsten rat sarcoma viral Oncogene (*KRAS*), resulting in the activation of Mitogen activated protein kinase (MAPK) occurs early in several cancer progression (Borras et al., 2011; Dolatkhah et al., 2015). In our previous work we showed that *KRAS* mutation had significantly associated with poor prognosis in CRCs (Dolatkhah et al., 2016).The purpose of this study is to investigate the following research question: In a Persian population at 2 urban hospitals what is the risk of genetic

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mutations associated with socioeconomic and lifestyle factors, and also dietary habits in CRC patients?

Materials and Methods

Patients and Design

We considered 147 patients with suspected CRC during colonoscopy from 2 most referent general hospitals (Imam Reza and Sina) of Tabriz, Northwest of Iran with Azeri ethnicity. The diagnosis of CRC was histologically confirmed by an expert pathologist. According to the inclusion and exclusion criteria reported in this research study, we included any cases with confirmed primary and sporadic colorectal cancer, with any morphologic types. We excluded those patients with any dysplastic polyps, secondary CRC, or hereditary CRC disease, and 100 patients were eligible for our investigation with confirmed colorectal cancer. All 100 patients were enrolled in this study for 2 years between 2013 and 2015.

Ethical Approval

The study protocol was approved by Ethics Committee of Tabriz University of Medical Sciences (Permit Number: 5.74.1235). Informed consent was obtained from all individual participants included in the study.

Molecular Tests

Sampling and testing were done as described in previous reports (Dolatkhah et al., 2016; Dolatkhah et al., 2017). Briefly, neoplastic and non-neoplastic tissue samples, measuring 2–4 mm, were obtained from the colon and rectum of patients during colonoscopy. These were sent to a molecular laboratory, coded, and stored at −80°C until testing. For molecular testing, we extracted genomic DNA from the tissue samples according to the manufacturer's guidelines (CinnaGen Company), and used them for PCR reactions with primers encompassing *KRAS* exon 2 (codon 12, 13) and BRAF exon 15 (codon 600) (Borras et al., 2011). Sanger sequencing was performed after purification with a PCR Product Purification Kit (MBST, Tehran, Iran), and sequencing was done with forward primers of PCR amplification, using an Applied Biosystems Genetic Analyzer (ABI 4-capillary 3130 Genetic Analyzer).

Measures

Data were retrieved from baseline survey of lifestyle factors, dietary behavior, and SES, as well as anthropometric evaluations during a physical examination of CRC patients. The lifestyle assessment measured 8 standard variables: smoking, drinking alcohol, eating breakfast, sleep time, physical exercise, work time, sedentary behavior, and life experiences .The lifestyle data were obtained during interview and by standard questionnaire according to references, and has been validated previously (Sarrafzadegan et al., 2009; Xu et al., 2012).

Alcohol consumption was assessed as never, low, moderate, and high levels of drinking of any type of alcohol per week (Xu et al., 2012). Diet was assessed in the context of the 5 main food groups, using Data Into Nutrients for Epidemiological Research (DINER) to evaluate the subjects' 7-day,24-hour dietary behaviors (Welch et al., 2001), and was validated during our previous study (Dolatkhah et al., 2016). The participants' intake of vegetables and fruits; meat, including red and white meat, fish, and processed meat; bread, including white, whole grain, and oat bread; other carbohydrates such as rice, macaroni, and sweets; and caffeine such as black tea, green tea, and coffee, were assessed as defined meals per day described as either weight or volume for each food groups.

The SES information was obtained using a researcher-designed questionnaire, which was validated during our previous study (Dolatkhah et al., 2016), and contains questions about residency, occupation, education, and income satisfaction. The response options designed as Likert scales and each variable scored from best to worth status. Subjects' educational were surveyed in categorical levels ranging from illiterate to college diploma or higher. Income satisfaction surveyed as monthly subjects' income, because the income amounts is not a good determinant for this topic in country. It was determined by patients' response to the question, "What is your income satisfaction level based on your total monthly family income from all sources?" The response ranged from satisfied to without satisfaction or without income. Patients were surveyed for occupation in categorical levels ranged as full time, part time, retired, and unemployed. The residency status of patients was defined as private or rental home, or unstable residence. The total scores for all criteria for each individual indicated the SES. The cut-points used for SES were considered as very good for 1–5, good for 6–10, moderate for 11–15, and bad for >15 scores.

Analytical Model

All analyses were performed using IBM SPSS, Version 19.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics (mean, standard error and percentage) were calculated to reflect the CRC patients' characteristics. Logistic regressions models were performed to assess the likelihood of presence of mutation in health-risk behaviors including lifestyle, dietary, and socioeconomic factors. In the first step of logistic regression, single association of each variable was analyzed separately, and the presence of mutations was considered to be dependent variables. Adjusted odds ratios (ORs) with 95% confidence intervals (CI) were constructed in the contexts multivariate analyses, with adjustment for age, sex, body mass index (BMI), marital status, and other mentioned variables. To obtain a more accurate regression analysis for some variables as work, sleep, smoking and alcohol consumption, and other lifestyle habits, we categorized these variables as acceptable numbers of each group. For example, circadian sleep variable was categorized as normal sleep pattern (6–8 h sleep) or abnormal sleep pattern (<6 or >8 h sleep).

Results

Study Participants Descriptive Characteristics

Of the 100 patients included in the study, 65 were men and 35 were women. The mean age of patients was

Table 1. Lifestyle, Dietary Habits and Socioeconomic Characteristics in *KRAS* Mutant and Wild Type CRCs

Factors	Groups	Mutant KRAS		Wild-Type KRAS	
		Number	Percent (within group)	Number	Percent (within group)
Sex	Male (64)	18	69.20	46	62.20
	Female (36)	8	30.80	28	37.80
Age	<50 years (20)	6	23.10	14	18.90
	≥50 years (80)	20	76.90	60	81.10
Marital Status	Married (78)	21	80.00	57	77.00
	Unmarried (22)	5	19.20	17	23.00
Circadian Sleep	Normal (69)	18	69.20	51	68.90
	Abnormal (31)	8	30.80	23	31.10
Sporting	Yes (28)	9	34.60	19	25.70
	No (72)	17	65.40	55	74.30
Work Status	<6hours/Day (66)	18	69.20	48	64.90
	≥6hours/Day (34)	8	30.80	26	35.10
Sedentary	Often or always (39)	11	42.30	28	37.90
	Rarely or Sometimes (61)	15	57.70	46	62.10
Life Satisfaction	Bad or Moderate (44)	12	46.20	32	43.20
	Good or Excellent (56)	14	53.80	42	56.80
Smoking	<1 Box†/day,< 1 Year (3)	2	7.70	1	1.40
	<1 Box/day,>1Year (15)	6	23.10	9	12.20
	>1Box/day,>1Year (10)	1	3.80	9	12.20
	Never/Others (72)	17	65.40	55	74.30
Drinking	Low (12)	5	19.20	7	9.50
	Moderate (7)	2	7.70	5	6.80
	Never (81)	19	73.10	62	83.80
BMI	≤25 (64)	18	69.20	46	62.20
	25-30 (36)	8	30.80	28	37.80
Vegetables and Fruits	≤2meal/day (51)	14	53.80	37	50.00
	≥ 3 meals/day (49)	12	46.20	37	50.00
Red Meat	Very Low (52)	16	61.50	36	48.60
	At least 1meal/day (48)	10	38.50	38	51.40
Bread	White Bread ≤1meal/day (31)	10	38.50	21	28.40
	White Bread ≥2 meals/day (67)	16	61.50	51	68.90
	Whole Grain (2)	-	-	2	2.70
Other Carbohydrates*	Eat (84)	24	92.30	60	81.10
	Not eat (16)	2	7.70	14	18.90
Tea	Black tea, 1-2 cups/day (43)	10	38.50	33	44.50
	Black tea, ≥3 cups/day (52)	14	53.80	38	51.40
	Green tea#/Non (5)	2	7.70	3	4.10
Marital Status	Married (78)	21	80.00	57	77.00
	Unmarried (22)	5	20.00	17	23.00
Residence	Private (88)	23	88.50	65	87.80
	Rental or Unstable (12)	3	11.50	9	12.20
Occupation	Full or Part Time (29)	11	42.30	18	24.40
	Retired or Unemployed (71)	15	57.70	56	75.60
Education	University or High School (33)	11	42.30	22	29.70
	Primary School or Illiterate (67)	15	57.70	52	70.30
Income Satisfaction	Satisfied (48)	16	61.60	32	43.30
	Unsatisfied (52)	10	38.40	42	56.70
Socio-Economic Status	Good (40)	15	57.69	25	33.78
	Moderate (59)	11	42.31	48	64.86
	Bad (1)	-	-	1	1.36

†Cigarette smoking; *Including rice, sweats, macaroni; # At least one meal/day

Table 2. Results of Univariate and Multivariate Regression Analyses of Life Style Factors with *KRAS* Mutation

Variable		KRAS Mutation		Unadjusted Regression			Adjusted Regression		
		Number	Percent	OR	95% CI		OR	95% CI	
					Lower	Upper		Lower	Upper
Sex	Male (64)	18	28.10	1.37	0.53	3.56	1.12	0.51	2.45
	Female (36)	8	22.20	Ref	-	-	-	-	-
Age	<50 years (20)	6	30.00	1.29	0.44	3.79	2.01	0.86	4.65
	≥50 years (80)	20	20.00	Ref	-	-	-	-	-
Marital Status	Married (78)	21	26.90	1.25	0.41	3.82	1.29	0.55	3.01
	Unmarried (22)	5	22.70	Ref	-	-	-	-	-
Circadian Sleep	Normal (69)	18	26.10	1.02	0.39	2.67	1.13	0.59	2.18
	Abnormal (31)	8	25.80	Ref	-	-	-	-	-
Sporting	Yes (28)	9	32.10	.65	0.25	1.71	1.90	0.94	3.82
	No (72)	17	23.60	Ref	-	-	-	-	-
Work Status	<6hours (66)	18	27.30	1.22	0.47	3.18	2.11	0.81	3.66
	≥6hours (34)	8	23.50	Ref	-	-	-	-	-
Sedentary	Often or always (39)	11	28.20	1.21	0.49	2.99	1.46	0.75	2.86
	Rarely or Sometimes (61)	15	24.60	Ref	-	-	-	-	-
Life Satisfaction	Bad or Moderate (44)	12	27.30	1.13	0.46	2.76	1.25	0.65	2.39
	Good (56)	14	25.00	Ref	-	-	-	-	-
Smoking	Yes (32)	9	28.10	1.17	0.46	3.02	1.91	0.47	7.70
	No (68)	17	25.00	Ref	-	-	-	-	-
Drinking	Yes (19)	7	36.80	1.90	0.66	5.52	1.85	0.80	4.29
	No (81)	19	23.50	Ref	-	-	-	-	-
BMI	≤25 (64)	18	28.10	1.37	0.53	3.56	1.42	0.72	2.80
	25-30 (36)	8	22.20	Ref	-	-	-	-	-

Notes, Lower, lower bound for 95% CI; upper, upper bound for 95% CI; variable adjusted for, age, sex, BMI, Smoking, Drinking and Marital Status; Abbreviations, OR Odds Ratio; CI Confidence Interval; Ref Reference

61.94 (±15.34) years, ranged from 23 to 90 years. Of the 100 patients, 20% (n = 20) were aged below 50 years. Moreover, the most common age group was the fifth decades of life (26% of patient with CRCs). The mean BMI of patients was 23.88 (±3.63), ranged from 15.24 to 33.31.

Twenty six (26%) cases had heterozygote mutant *KRAS* of which 16 patients had mutations in codon 12, nine in codon 13, and in one patient it was detected in codon 10, which was reported previously (Dolatkhah et al., 2017). BRAF mutations were not detected in the amplified exon in any of the studied cases.

Males smoked more (43.8% of males vs. 11.1 % of females) and alcohol consumption, which has been mentioned by 19% of patients, was more by men (28% and 2.8% respectively). Males had more sporting and working times, and had better life satisfaction according to SES questionnaire.

The use of black tea is historically and culturally very common among our population; so 95% of the patients in the study reported drinking black tea, with a frequency of at least 3 cups/day in 52% of them. In total, 78 of our patients were married, and most of them (88%) had a private home. Most of the patients were not satisfied with their income or had no income (52%), of whom the majorities were female. SES was very good or good in 40% of patients, 59% of them had a moderate SES, and only 1 patient reported a bad SES (Table 1).

Analytical Statistics Related to KRAS Mutation

Fully adjusted model regression analysis showed that sedentary lifestyle had a direct impact on mutation, so that patients with more often or always sedentary, was more likelihood of *KRAS* gene mutation (OR: 1.46; 95% CI: 0.75–2.86). Patients who worked <6 h/day were more likely to mutation, with an adjusted OR of 2.11 (95% CI: 0.81–3.66). Alcohol consumption was associated with higher likelihood of *KRAS* gene mutation (OR: 1.85; 95% CI: 0.80–4.29) (Table 2).

Patients consuming low red meat (but not processed or grilled meats) had more likelihood of mutation (OR: 2.29; 95% CI: 1.15–4.55) , and carbohydrates intake, such as rice, macaroni, and sweets, at least once-daily increased the likelihood of mutation about 4 times (OR: 3.56; 95% CI: 1.13–11.21). The likelihood of *KRAS* gene mutations in the patients who drank at least 3 cups of black tea daily were 17% higher , but this was not statistically significant (OR:1.17; 95% CI: 0.56-2.43) (Table 3).

Fully adjusted regression analysis showed that more highly educated patients (high school or university education) had decreased odds of a *KRAS* mutation (OR:0.80; 95% CI:0.31-2.05). None of the above-mentioned socioeconomic variables had

Table 3. Results of Univariate and Multivariate Regression Analyses of Dietary Factors with KRAS Mutation

Variable		KRAS Mutation		Unadjusted Regression			Adjusted Regression		
		Number	Percent	OR	95% CI		OR	95% CI	
					Lower	Upper		Lower	Upper
Sex	Male (64)	18	28.10	1.37	0.53	3.56	1.45	0.40	5.31
	Female (36)	8	22.20	Ref	-	-	-	-	-
Age	<50 years (20)	6	30.00	1.29	0.44	3.79	0.66	0.27	1.64
	≥50 years (80)	20	20.00	Ref	-	-	-	-	-
Vegetables and Fruits	≤2meal/day (51)	14	27.50	1.17	0.48	2.86	1.15	0.59	2.24
	≥ 3 meals/day (49)	12	24.50	Ref	-	-	-	-	-
Red Meat	Very Low (52)	16	30.80	1.69	0.68	4.21	2.29	1.15	4.53
	At least 1meal/day (48)	10	20.80	Ref	-	-	-	-	-
White Bread	≤1meal/day (31)	10	32.30	1.52	0.60	3.88	1.91	0.93	3.91
	≥2 meals/day (67)	16	23.90	Ref	-	-	-	-	-
Other Carbohydrates	Eat (84)	24	28.60	2.8	0.59	13.26	3.56	1.13	11.21
	Not eat (16)	2	12.50	Ref	-	-	-	-	-
Black Tea	1-2 cups/day (43)	10	23.30	Ref	-	-	-	-	-
	≥3 cups/day (52)	14	26.90	1.22	0.48	3.10	1.17	0.56	2.43
BMI	≤25 (64)	18	28.10	1.37	0.53	3.56	1.10	0.51	2.37
	25-30 (36)	8	22.20	Ref	-	-	-	-	-

Notes, Lower, lower bound for 95% CI; upper, upper bound for 95% CI; variable adjusted for, age, sex, BMI, and intake of Bread, Vegetables and Fruits, Tea, Carbohydrate, Red Meat; Abbreviations, OR Odds Ratio; CI Confidence Interval; Ref Reference

Table 4. Results of Univariate and Multivariate Regression Analyses of Dietary Factors with KRAS Mutation

Variable		KRAS Mutation		Regression Analysis Unadjusted			Regression Analysis Adjusted		
		Number	Percent	OR	95% CI		OR	95% CI	
					Lower	Upper		Lower	Upper
Sex	Male (64)	18	28.10	1.37	0.53	3.56	1.11	0.50	2.46
	Female (36)	8	22.20	Ref	-	-	-	-	-
Age	<50 years (20)	6	30.00	1.29	0.44	3.79	1.17	0.51	2.68
	≥50 years (80)	20	20.00	Ref	-	-			
Marital Status	Married (78)	21	26.90	1.25	0.41	3.82	0.83	0.35	2.00
	Unmarried (22)	5	22.70	Ref	-	-	-	-	-
Residence	Private (88)	23	26.10	1.06	0.26	4.26	0.87	0.31	2.42
	Rental or Unstable (12)	3	25.00	Ref	-	-	-	-	-
Occupation	Full or Part Time (29)	11	37.90	2.28	0.89	5.85	0.96	0.33	2.81
	Retired or Unemployed (71)	15	21.10	Ref	-	-	-	-	-
Education	University or High School (33)	11	33.30	1.73	0.69	4.37	0.80	0.31	2.05
	Primary School or Illiterate (67)	15	22.40	Ref	-	-	-	-	-
Income Satisfaction	Satisfied (48)	16	33.30	0.48	0.19	1.19	1.05	0.41	2.69
	Unsatisfied (52)	10	19.20	Ref	-	-	-	-	-
Socio-Economic Status	Good (40)	15	37.50	2.62	1.05	6.54	3.09	1.38	6.95
	Moderate (59)	11	18.60	Ref	-	-	-	-	-
	Bad (1)	-	-	-	-	-	-	-	-

Notes, Lower, lower bound for 95% CI; upper, upper bound for 95% CI; variable adjusted for, age, sex, Marital, Residence, Occupation, Education, and Income Satisfaction; Abbreviations, OR Odds Ratio; CI Confidence Interval; Ref Reference

significant association with *KRAS* gene mutation after adjusting for sex, age, and marital status.

In general, based on their socioeconomic variable scores, the patients could be categorized in 4 groups: very good, good, moderate, and poor SES. Only one female patient had poor SES, and that same patient did not have any mutation. The CRC patients in good SES had odds of mutation that were 3.01 times higher than the other groups (OR: 3.01; 95% CI: 0.69–13.02) (Table4).

Discussion

To the best of our knowledge, this is the first study in country seeking to identify the relationship between lifestyle and nutritional factors, and SES with the occurrence of specific gene mutations involve in CRC. However, we tried to get more realistic evidences from SES of CRC patients in this survey that was a valuable and interesting point in this study. The respective questionnaires have contained questions which were derived from studies of relevant references, and have been already validated (Dolatkhah et al., 2016). The small sample size in this study were among the main constrains, due to technical difficulties of collecting fresh tissue samples from newly diagnosed CRCs. Also we just performed the double sequencing for *KRAS* positive samples, to ensure the accuracy of the tests, because of limitations of time and budget.

In a case study conducted on 108 patients suffering from sporadic CRC, a relationship among high unsaturated fat levels, low calcium levels, and an increase in *KRAS* gene mutations was reported (Bautista et al., 1997; Slattery et al., 2000; Weijenberg et al., 2007; Naguib et al., 2010; Brandstedt et al., 2014). In another study conducted by Martinez et al., there was no relationship between various mutations and nutritional factors (Martinez et al., 1999). In a study on a large number of nutritional factors, using 1428 CRC patients, a significant relationship between vegetable consumption and *KRAS* gene mutations was found. Those who consumed fewer vegetables suffered more mutations. High carbohydrate and refined grain intakes were also related to a higher risk of *KRAS* gene mutations, particularly at codon 13. In addition, in this study, it was observed that the consumption of processed meats was related to certain mutations of *KRAS* gene (Slattery et al., 2007). Although there were conflicting findings about the association of red meat intake and the risk of colorectal cancer (Potter, 2017), but it is clear that cooking methods and the type of meat (proceed meat) are the major factors which associate with the risk of any mutation in CRC. Also, important measurable nutrients found in meat (especially Iron and fat) have recently received considerable attention in association with specific mutations involve in molecular pathways of colorectal carcinogenesis. Gilsing et al., (2013) specifically observed a dose-response relation between heme Iron intake and activating G>A mutations in *KRAS* gene, and an overall G>A mutations in APC gene. By adjusting the association for total meat intake we found contradictory results in our survey. Patients with very low intake of red meat had more likelihood of mutation than patients who had at least one meal of meat per day (OR: 2.29; 95% CI: 1.15–4.53). This may be because of the different cooking methods which are common in Iran (not processed or grilled meats) and different types of meat consumption in the country (with mostly lamb and sheep , and lesser beef, veal, and pork).

The relationship between anthropometric factors and mutations of *KRAS* was investigated in a study conducted in Sweden in 2014. High weight-to-height ratio and BMI and obesity were related to increased odds of *KRAS* gene mutations. Interaction analysis showed a significant difference between men and women in terms of the BMI *KRAS* mutation, where men showed a stronger relationship (Brandstedt et al., 2014). Because *KRAS* gene mutations occur at the primary stages of CRC carcinogenesis, it seems that obesity biologically influences the risk of mutations related to tumor status. In our study, there was no meaningful relationship found between the BMI level and *KRAS* gene mutation occurrence, but each unit of BMI increase was associated with a 3% higher likelihood of *KRAS* gene mutation. The potential relationship among BMI, overweight, and genetic mutations demands further research.

According to epidemiological population-based study results, a clear relationship has been found between the levels of tobacco and alcohol consumption and the risk of CRC. However, few studies have been conducted on the molecular relationship among smoking, alcohol use, mutations, and CRC pathogenesis. Smoking has been linked to an increased occurrence of CpG island methylator phenotype high (CIMP-high) tumors, and along with alcohol consumption and DNA mutation, epigenetic changes in CRC. In addition to the fact that alcohol consumption (particularly beer) has been related to increased CRC risk, it has been recognized that high levels of alcohol consumption and poor diet in terms of folate, B12, and B6 affects DNA methylation abnormalities and increases the likelihood of CpG island methylator phenotype cancers (Samowitz et al., 2006; Slattery et al., 2007). Based on logistic regression analysis results in the present study, alcohol consumption (even at medium or low levels) was associated with about double the likelihood of *KRAS* gene mutations. On the other hand, smoker CRCs had 28% higher odds of the *KRAS* gene mutation compared to non-smokers.

A sedentary lifestyle with low activities will also increase BMI. A close relationship has been observed between the stage and grade of CRC and increases in the hours spent in sedentary leisure activities over 6 h/day (Boyle et al., 2014; Yu et al., 2014). However, it appears that there has not been any comprehensive study on the relationship between sedentary lifestyle and genetic mutations. Only one study in China has investigated the relationship between lifestyle and the levels of certain molecular biomarkers related to mRNA. The study showed that a sedentary lifestyle has a direct relationship with the levels of these biomarkers, where more hours without mobility were associated with higher levels of these biomarkers (Yu et al., 2014).

The most interesting finding of our analysis was that the amount of sedentary lifestyle activities had a direct effect on the odds of *KRAS* gene mutations, so that a low-activity or completely sedentary lifestyle increases the likelihood of *KRAS* gene mutation by about 50%, in line with previous observations (Boyle et al., 2011). According to our results those patients who worked more than 6hours per day had 2 times lower likelihood of *KRAS* mutations. Therefore, the data suggests that programs to control and prevent CRC should emphasize increasing physical activity and keeping body weight within a healthy range (Boyle et al., 2011; Yu et al., 2014).

The present research found different relationships between SES and CRC molecular pathways compared to previous studies. We found that patients with very good or good SES had 3 times higher likelihood of *KRAS* gene mutation. This study and other research have suggested that there are greater CRC risks among people in higher socioeconomic classes, due to the increases in encountering risk factors such as a sedentary lifestyle, alcohol and tobacco consumption, high-calorie diets, and more red meat consumption (Faggiano et al., 1997; Rohani-Rasaf et al., 2013). Further studies are warranted regarding whether this exposure could explain the higher chances for genetic mutations among these individuals.

In conclusion, it is critical to plan CRC screening and prevention programs based on the most prominent risk factors for the disease and on these factors' interactions with genetic mutations. For CRC, these factors have been identified as including a person's lifestyle, dietary habits, environment, and cultural factors.

Most deaths resulting from CRC can be prevented. The prevention strategies for this form of cancer include an appropriate diet, increasing physical activity, and attaining and staying at a healthy weight. Of course, research has not yet described the exact relationship among genetic mutations, such as those affecting *KRAS* and BRAF, environmental and nutritional risk factors, and the stage and prognosis of CRC. Lastly, based on this study's support for a relationship between the frequency of *KRAS* gene mutations and factors such as race, ethnicity, geographic location, lifestyle, and diet, future studies with larger sample sizes are necessary to further explore these concepts.

Author contributions

RD

Substantial contributed to conception and design of the study, acquisition of data, analysis and interpretation of data, drafted and wrote the article, did the final approval of the manuscript, agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

MHS

Substantial contributed to conception and design of the study, drafted and revised the article, did the final approval of the manuscript, agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

IAK, RS, and FF: substantial contributed to conception and design of the study, revised the article, did the final approval of the manuscript, agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

AF and AD

Substantial contributed to conception and design of the study, analysis and interpretation of data, revised the article, did the final approval of the manuscript, agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Disclosure

The abstract of this paper was presented at the IARC @ 50 , Global Cancer Occurrence , Causes, and avenues to Prevention 2016, as a poster presentation with interim findings. The poster's abstract was published in "Poster Abstracts" in IARC Abstract Book, available from: www.iarc-conference2016.com.

Conflicts of interest

The authors report no conflicts of interest in this work.

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