Corrections requested by EDITOR

Grant: The Approved Grant Application Form(s) or Funding Agency Copy of any Approval Document(s)", which consists of several pages, is attached below the first page on which it is written "Supported by grant from São Paulo Research Foundation (FAPESP) (grant 2011/23969-1)Grant term - São Paulo Research Foundation (FAPESP) process number 2011/23969-1 ".

Changes made in the manuscript

■ **(Fifth page) In the aim of the abstract the words were reduzidasd para 20 words as we can see below.**

To evaluate the association of polymorphisms in GSTs in the risk of SCRC, tumor progression and survival of patients.

■ **(Fifth page) Words were added in the abstract methods section as we can see below.**

METHODS

A case-control study was conducted in 970 individuals from the Brazilian population (232 individuals from the case group with colorectal cancer and 738 individuals from the control group without a history of cancer). PCR multiplex and PCR-RFLP techniques were used for genotyping of the polymorphisms. The tumor was classified according to TNM: tumor extension (T), affected lymph nodes (N), and presence of metastasis (M). logistic regression, multiple logistic regression and survival analysis were used to analyze the data. The results were presented in terms of odds ratio (OR) and 95% confidence interval (CI). The level of significance was set at 5% ($P \leq 0.05$).

■ **(Eighth page, fourth paragraph) Added in the introduction more details about the GST as we can see below.**

They catalyze the conjugation of structurally different by-products of oxidative stress and xenobiotics to glutathione (GSH), which leads to eliminate toxic substances from the cells and protecting important cellular components like nucleic acids and proteins[12].GST gene expression varies between different tissues and cell types[13].

In addition to being very common in the general population, the complete absence of GSTT1 and / or GSTM1 may alter their expression or the activity of the protein itself[14]. And in general, GSTP1 appears to be highly expressed in proliferating cells than in differentiated cells. In addition, many of the GSTs are overexpressed in various neoplastic cells and higher levels are observed in aggressive cancer cells[15]. The change in the GSTP1 gene also significantly alters the enzymatic activity[16,17], influencing in the detoxification of carcinogens, causing DNA damage, exerting an indirect effect on the risk of cancer development[18].

■ **(Twelfth page) The results were subdivided as we can see below.**

RESULTS

Sociodemographic data

Table 1 presents the demographic data of SCRC patients and controls. Age equal to or above 62 years (OR = 8.79; 95% CI = 5.90-13.09; P < 0.01) and female gender (OR = 2.91; 95% CI = 1.74-4.37; P < 0.01) were associated with risk of SCRC. The genotypic frequencies of GSTP1 Ile105Val polymorphism were in HWE in both groups (Case: P = 1, Control: P = 0.29).

Individual polymorphism analyzes

GSTM1 null genotype carriers presented a higher risk of developing the disease (OR = 1.45; 95% CI = 1.06-2.00; P = 0.022). On the other hand, the GSTT1 polymorphism was associated with a reduced risk of SCRC (OR = 0.65; 95% CI= 0.43-0.98; P = 0.037; Table 1).

In the present study, there was a significant interaction between the presence of the wild-type allele of the GSTP1 Ile105Val polymorphism and smoking habit in the risk of SCRC (OR = 2.33; 95% CI = 1.34-4.05; P = 0.049). However, there was no interaction between the other polymorphisms and smoking or drinking habits in the risk of the disease (Table 2).

Regarding the clinical-histopathological parameters of the SCRC samples, the rectum was the most frequent primary site (60%), in addition to aggressive

tumors (69.65%; Table 3). There was only association between the GSTM1 null genotype and the presence of aggressive tumors (OR = 2.33, 95% CI = 1.23-4.41, P = 0.0087).

Analysis of the combined polymorphisms

An increased risk of SCRC was observed in the presence of the combination of the GSTT1 non-null/GSTM1 null genotypes (OR = 1.50; 95% CI

= 1.03-2.19; P = 0.033) and the GSTT1 non-null/GSTM1 null/GSTP1 Val* (*with the presence of at least one polymorphic allele) (OR = 1.85; 95% CI = 1.01-3.36; P = 0.045). The combined GSTT1 non-null/GSTM1 null genotypes (OR = 2.40; 95% CI = 1.19-4.85; P = 0.015) and GSTT1 non-null/GSTM1 null/GSTP1 Val* (OR = 2.92; 95% CI = 1.05-8.12; P = 0.040) were associated with tumor progression (Table 4).

Survival analysis

Kaplan-Meier curve analysis showed that the survival time of carriers of the polymorphic allele GSTP1 Ile105Val, and the GSTM1 and GSTT1 null genotypes, were not significantly different from the survival time of non- carriers of these polymorphisms (Table 5).

■ **(Eighteenth page, twelfth paragraph) References were added on the association of tumor progression with GST in the discussion as we can see below.**

Regarding the clinical-histopathological parameters of SCRC, **some studies have been showed that low activity GST genotypes can be associated with more aggressive tumour and survival in colorectal cancer patients[51,52].** iIt is possible to observe an association between the GSTM1 null genotype and the presence of advanced tumors. One study demonstrated an association between aggressive tumors with the presence of the GSTT1 null genotype[47]. However, other studies that evaluated the same polymorphisms did not find an association between the polymorphic genotypes and the clinical-histopathological parameters of SCRC[3,27,42,49].

- **And thirteenth paragraph was also been added as we can see below.**

This biological relationship between GST and progression is still not well described. But the possible explanation would because GSTs have play important roles in regulation of genes related with activating cellular maintenance, proliferation and apoptosis evasion. Thus, GSTs plays interacting with TNF receptor associated factor 2 (TRAF2) and decreasing signal

transduction from receptors in the tumour necrosis factor alpha-like (TNF-α) and c-Jun NH2-terminal kinase (JNK kinase) pathways[53-55].

■ **The tables that were repeated were deleted.**

Answering Reviewers

Firstly, we greatly appreciate the suggestions and criticisms of the both reviewers. They were very important contributing to improve our article. Below are the answers to the reviewers.

Reviewer #1: Cancer disease is a multifactorial one, as it was stated. The GST polymorphisms were much studied in the last years and the present paper found an increased risk for SCRC related to some of them. It would be interesting to know also the races+/- ethnicities involved in the study. The study had a large amount of controls. It was interesting that only women over 62 years old were more susceptible to SCRC, also related to GSTM1 null genotype, knowing that these polymorphisms are constitutional.

Answer: The brazilian population is quite micigenated therefore is not safe to establish a specific ethnicity or race.

Reviewer #2: The authors investigated the polymorphisms of glutathione S transferase (GST) superfamily in 232 cases of sporadic colorectal cancer (SCRC) and statistically analysed the association of clinicopathologic factors. The design of study itself is very interesting but I have some major comments.

- The more detailed information of GST superfamily and the rationale reason why these polymorphisms could be the risk factor of SCEC should be documented in INTRODUCTION section.

Answer: In the fourth paragraph of the INTRODUCTION section page 8 we added more information about the GSTs.

catalyze the conjugation of structurally diff erent by-products of oxidative stress and xenobiotics to glutathione (GSH), which leads to eliminate toxic substances from the cells and protecting important cellular components like nucleic acids and protein [Hayes et al]. GST gene expression varies between

different tissues and cell types [Rowe et al.,1997]. In addition to being very common in the general population, the complete absence of GSTT1 and / or GSTM1 may alter their expression or the activity of the protein itself [Economopoulos et al.,2010]. In general, GSTP1 appears to be highly expressed in proliferating cells than in differentiated cells. In addition, many of the GSTs are overexpressed in various neoplastic cells and higher levels are observed in aggressive cancer cells [Oguztuzun et al., 2011]. The change in the GSTP1 gene also significantly alters the enzymatic activity [Hezova et al., 2012; Tew et al., 2011], influencing in the detoxification of carcinogens, causing DNA damage, exerting an indirect effect on the risk of cancer development [Gong et al., 2012].

- The authors should divide the RESULTS section into several parts with subheadings.

Answer: As suggested by the reviewer, the topics "Sociodemographic data", "Individual polymorphism analyzes", "Analysis of the combined polymorphisms"and "Survival analysis" were added in the Results section.

- In the analysis of Table 1, the authors compare the age of SCRC group and Control group by the cut off of 62yrs. What is the rationale of this cut off? Furthermore, is this comparison reasonable?

Answer: The cutoff of <62yrs was used as reference because is mean of the amostral group after the normality test have been performed. The analysis in question is the result of a multiple logistic regression where the other variables are adjusted. This is important because cancer is a multifactorial disease and we want to evaluate all risk factors as well as polymorphisms. Others articles also used this analysis [Piao et al., 2009; Laï JL et al.,1995; Ma JY et al., 2018; Qu K et al., 2015; Pandith AA et al., 2013].

- The authors should document the mean (or median) and SD of age in these two groups.

Answer: About this suggestion was added in table 1 the values of mean and Standard Deviation of age of both groups.

- I can understand relationships of GST superfamily polymorphisms and carcinogenesis but I cannot understand the why these polymorphisms correlate the tumor progression (Table 4). Are the pathological factors, such as tumor differentiation, lympho-vessel invasion, or lymph-node metastasis different by polymorphisms?

Answer: We added in the fourth paragraph of the INTRODUCTION section page 8 a sentence about the possible influence between GST and tumor progression.

"In general, GSTP1 appears to be highly expressed in proliferating cells than in differentiated cells. In addition, many of the GSTs are overexpressed in various neoplastic cells and higher levels are observed in aggressive cancer cells."

We added in the SECOND and TIRTHY paragraph of the DISCUSSION section page 18 a sentence about the possible influence between GST and tumor progression.

"Have been showed that low activity GST genotypes can be associated with more aggressive tumour and survival in colorectal cancer patients (Mitsudomi et al., 2000; Holley et al., 2006)."

"This biological relationship between GST and progression is still not well described. But a possible explanation would be because GSTs have play important roles in regulation of genes related with activating cellular maintenance, proliferation and apoptosis evasion. Thus, GSTs plays interacting with TNF receptor associated factor 2 (TRAF2) and decreasing signal transduction from receptors in the tumour necrosis factor alpha-like (TNF-α) and c-Jun NH2-terminal kinase (JNK kinase) pathways [Hayes et al., 2005; Wu et al., 2006; Adler et al., 1999]."

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