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RESEARCH ARTICLE

RISK OF COLORECTAL CANCER AND CLOTTING FACTOR GENE POLYMORPHISMS IN MOROCCAN POPULATION.

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Abstract

Background: Venous thrombosis has been described as a common complication for cancer patients. The association between clotting factor gene polymorphisms and the risk of colorectal cancer has been evidenced. The aim of the present study was to investigate the association of G20210A factor II prothrombin (FII) and factor V Leiden (FVL) G1691A with the risk of colorectal cancer(CRC).*Methods and results*. Genotyping of FVL and G20210A FII was performed using the polymerase chain reaction restriction fragment length polymorphism method on a sample of 76 patients with CRC as well as 182 controls. No significant difference in FVL gene variations was observed between cases and controls. However, with regard to the G20210A FII , the homozygous mutated genotype AA was associated with an increased risk of CRC. A significant association between the G20210A FII mutation and the risk of CRC was identified using recessive (OR=57.63, 95% CI: 3.33-997.26, P=0.0053), dominant (OR=27.87, 95% CI: 12.67 -61.28, P<0, 0001) and additive (OR=21.24, 95% CI: 10.45-43.16, P<0, 0001) models. No statistical difference was observed in parameters such as sex, age and positive family history for cancer .*Conclusion*. Our results did not support an effect of FVL gene on CRC risk and suggested that the G20210A FII prothrombin gene variant may be a risk factor for CRC in Moroccan population.

Introduction:-

Thromboembolic venous disease and cancer are two commonly entangled pathologies. 20% of patients with cancer will also have thromboembolic disease venous [1]. This is the second cause of death during the cancerous disease and constituting a factor of poor prognosis with a decrease in survival [2]. It was demonstrated that inaugural thrombosis can reveal cancer [3]. Actually, the link between hemostasis , thrombosis and development neoplasia is more clearly established, in particular with the tissue factor (TF), which is the determinant of thrombogenicity induced by cancerous disease [4,5] . This TF expressed by tumors cells is a physiological stimulus of coagulation. It activates the coagulation cascade and leads to the synthesis of thrombin and next formation of the fibrin network

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which constitutes a proangiogenic matrix facilitating vascular infiltration [6,7]. In addition recently, it has been shown that, under the influence of coagulation factors, peritumoral macrophages favored migration and tumor invasion [8]. Some types of cancer are more often associated with thrombosis such as the pancreas, lung, prostate, ovary, colorectal cancer (CRC), acute promyelocytic leukemia and myeloproliferative syndromes [3].

CRC is a major public health problem in the world. It is the third most common cancer in Morocco [10]. Sporadic CRC are a group of heterogeneous malignant disorders because they are determined by both genetic and environmental factors [11]. The CRC has been the subject of numerous genetic studies in recent years, with the aim of finding genetic tests strong enough to enable early diagnosis or primary screening of this disease, as well as a better understanding and use of therapeutic means.

Although the interactions between thrombosis and colorectal cancer is well established. The two most common prothrombotic mutations are Factor V Leiden (FVL) and Prothrombin G20210A factor II Prothrombin (FII) [11, 12]. the FVL , an substitution of arginine by glutamine at amino acid position 506 of coagulation factor V, is responsible for protein C resistance, who is likewise an major risk factor for venous thrombosis [13]. Also G20210A FII , an substitution of guanine by adenine at position 20210 on chromosome 11 (11p11) in a 3 'untranslated region of the prothrombin gene , is a precursor of thrombin which activates factors V and VIII and converts fibrinogen to fibrin who plays a major role in the formation of thrombosis [14] . Some studies have demonstrated that FVL and G20210A FII polymorphisms have been associated with tract digestive cancer. The number of available studies on the variations of coagulation factor gene polymorphisms in patients with colorectal cancer is limited and their interpretation has been controversial [15, 16, 17]. In the present study, the main aim was to investigate the association of the G1691A FVL and G20210A FII with CRC among Moroccan patients.

Materials and Methods:-

Study Population:-

The study population consisted of 258 subjects, including 76 patients with histologically confirmed CRC, recruited at the Department of Visceral Surgery of CHU Ibn Rochd (Casablanca, Morocco) and 182 healthy control subjects recruited from volunteer blood donors with no malignant pathology. Clinical parameters such as age, gender, family antecedents, and time to diagnosis were also collected. This study was approved by the Ethics Committee of Hassan II University (Casablanca, Morocco). Informed consent was obtained from all participants before blood sampling and genetic analysis.

DNA Extraction:-

DNA was extracted from 5-ml blood sample using the salting out procedure [18]. The quality and quantity of the DNA were checked using a spectrophotometer.

Genotype Determination:-

Genotyping of the G1691A FV and G20210A FII mutations were detected by polymerase chain reaction (PCR) and restriction fragment length polymorphism analysis as previously described by Huber et al [19] and Danneberg et al[20] respectively.

Amplified fragments were cleaved using Hind III restriction enzyme for both polymorphisms. The digested product was electrophored on 3% agarose gel and stained with Ethidium Bromide and visualized with ultraviolet rays using transilluminator.

Digestion of the amplified fragment at the Hind III site produced fragments of 209 and 32 bp and fragments of 322 and 23 bp for FVL and G20210A FII respectively. Wild type remains intact for both [20, 21].

Statistical Analysis:-

Statistical analysis was performed using SPSS 21.0 software. Hardy–Weinberg equilibrium (HWE) test was performed separately for cases and controls group. The clinical and histological features and the relation to different genotypes were made by chi-squared ($\chi 2$) test. Associations between genotypes and CRC risk were assessed by calculating odds ratio (OR) with confidence intervals (CI) of 95%. Significance was approved at value less than 0.05.

Results:-

Characteristics of the Study Population:-

The study cohort consisted of 76 patients with CRC and 182 apparently healthy controls. The average age of patients was 48 years (range, 18-79 years).The distal location of the tumor (left colon, sigmoid and rectum) was predominant with 79% of cases.

Correlation analysis for the polymorphisms:-

FVL mutations:-

FVL mutations of the homozygous or heterozygous genotype were not detected in any of the cases or the controls. All the subjects were found to be normal homozygous.

G20210A FII mutations:-

The distribution of the G20210A FII mutation was within the Hardy-Weinberg Equilibrium (HWE) in the cases and controls (table 1). The genotype frequencies of G20210A FII in CRC patients were 38.1% GG (wild-type), 48.7% GA (heterozygous mutated type) and 13.2% AA (homozygous mutated type), and those in the control subjects were 94.5% GG, 5.5% GA and 0.0% AA. The allele frequencies were 62.5% G and 36.5% A in the CRC patients vs. 97.3% G and 2.7% A in the controls (Table 2). Statistical analysis revealed that the GA (heterozygous mutated type) and the AA-mutated homozygous type of G20210A FII was significantly associated with an increased risk of CRC (OR = 21.9 95%CI=9.84-48.92, P<0,0001) and (OR =122.7 95% CI:7.00-2152.45, P=0,001) and respectively ((Table 2). Using three models of genotypic combination, a significant association with the risk of CRC was determined with the recessive model (AA vs. GG+GA; OR=57.63, 95% CI: 3.33-997.26, P=0.0053), the dominant model (GA+AA vs. GG; OR= 27.87 , 95% CI: 12.67 -61.28, P<0,0001) and the additive model (A vs. G OR=21.24, 95% CI: 10.45-43.16, P<0,0001) (Table 2).

No positive correlation was observed between G20210A FII mutation and patient age, sex, family history, diagnostic delay, location, histology and stage of the tumor (table3).

Table1:- HWE among cases and controls.

genotype	HWE cases		HWE controls	
	X^2square	P value *(p>0.05)*	X^2 square	P value (p>0.05)
G20210A FII	0.113	**0.7365***	0.145	**0.7031***
*Statistically significant				

Table 2:- Genotypic and allelic frequencies of the *G20210A FII* polymorphism in colorectal cancer patients and controls

G20210A FII genotypes/ alleles	Cases (%)	Controls %	OR (95% CI)	P value
GG	38.1	94.5	1	
GA	48.7	5.5	21.9 [9.84-48.92]	**<0,0001***
AA	13.2	0	122.7[7.00-2152.45]	**0,001***
GG+GA[(b)]	86.8	100	1	
AA	13.2	0	57.63 [3.33-997.26]	**0.0053***
GG[(c)]	38.1	94.5	1	
GA+AA	61.8	5.5	27.87[12.67 -61.28]	**<0,0001***
G[(d)]	62.5	97.3	1	
A	37.5	2.7	21.24 [10.45-43.16]	**<0,0001***

*Statistically significant.
[b] Recessive model.
[c] Dominant model.
[d] Additive model.

Table 3:- Genotypic frequencies of G20210A FII polymorphism in patients with colorectal cancer according to clinical and histological parameters.

	Number	G20210A FII				Chi-square Test
		GG (%)	GA (%)	AA (%)	P value	
Age					0.14	3.90
<45 years	34	35.3	58.8	5.9		
≥45 years	42	40.5	40.5	19.0		
Gender					0.46	0.80
male	40	35.0	50.0	15.0		
female	36	41.7	47.2	11.1		
Time to diagnosis <6 month					0.50	1.40
≥6 month	42	33.3	54.8	11.9		
	34	44.1	41.2	14.7		
Family Antecedent					0.70	2.18
CRC	12	33.3	58.3	8.3		
Other cancers	5	60.0	20.0	20.0		
No family history	59	37.3	49.2	13.6		
Localization					0.14	12.25
Right Colon	14	64.3	28.6	7.1		
Transverse Colon	2	50.0	50.0	0		
Left Colon gauche	16	25.0	62.5	12.5		
Sigmoid	28	42.9	50.0	7.1		
Rectum	16	18.8	50.0	31.2		
Histology component					0.60	1.01
ADK simple	59	37.3	47.5	15.3		
ADK colloid mucinous	17	41.2	52.9	5.9		
Differentiation					0.27	5.16
Well	27	40.7	37.0	22.2		
Moderate	36	33.3	55.6	11.1		
Poor	13	46.2	53.8	0		
Stage					0.13	9.90
Stage I	11	45.5	27.3	27.3		
Stage II	21	38.1	57.1	4.8		
Stage II	28	39.3	39.3	21.4		
Stage IV	16	31.2	68.8	0		

*Statistically significant

Discussion:-

The CRC is the first cancer of the digestive tract in Morocco [9]. The etiopathogenesis of sporadic CRC is still poorly understood. However, several studies have demonstrated the association of genes predisposing to a high risk or decreased occurrence of CRC. A relationship between different types of thrombotic gene polymorphism , including FV and FII, with CRC has been established. However, this was contradictory.

To the best of our knowledge, this is the first study to explore the potential association of the G20210A FII and FVL polymorphisms with CRC in the Moroccan population.

The association of FVL genotype with digestive cancer was previously demonstrated [15]. FVL homozygous carriers were shown to be increased risk for colorectal cancer in a German population [22]. In addition a significant effect of FVL on thrombosis in patients with malignant diseases (most patients with adenocarcinoma of the colon) was established by Pihusch et al [17] .Our study did not identify such a relationship with CRC . These results could be explained partly by a different ethnic context. Furthermore, our data are in agreement with the results by Paspatis et al [23] who did find no significant differences in the frequency of the FVL mutation of the prothrombin gene

compared to colonoscopically selected controls. Similarly, Ozkan et al did not report an association between FVL mutations and cancer patients [24] . Regarding the present results, no statistically significant association was observed between the FVL polymorphism and CRC in Morocco. However, Studies on larger populations need to test this null association.

The G20210A FII gene polymorphism is correlated with higher plasma levels of prothrombin among subjects wearing this variant compared to normal subjects, which makes them prone to thromboembolic disease [25, 26]. A high incidence of gastrointestinal neoplasia in men with persistent activation of the coagulation pathway was demonstrated by Miller et al [16]. Regarding the correlation between G20210A FII polymorphism and clinical features, we found that this polymorphism would have no impact on clinical events and histological tumor and may not be predictive factor of these parameters. This finding is consistent with what has been described by Paspatis et al [23]. A significant association of the mutated AA genotype and the risk of CRC was identified using three models of genotypic combination (recessive, dominant and additive). In addition, The A allele was statistically significant in our study and the persons with at least one 20210A allele are probable to develop CRC. Our data are in agreement with the findings by Pihusch et al [17] who indicate that the prothrombin G20210A mutation may be a possible cofactor in cancer pathogenesis but not with the results published by Vossen et al who reported that heterozygous mutated genotype (GA) reduced risk for colorectal cancer in a German population [22] .Furthermore, Paspatis et al and Ozkan et al reported no significant association between the prothrombin G20210A mutations and CRC [23,24]. The present results show that the G20210A polymorphism of the FII prothrombin gene was significantly associated with CRC. The results of the previous studies regarding this association remain inconsistent .These conflicting results could be explained by various factors including the mode of living, differences in genetic background among the studied populations; differences in the selection of patients and controls; studies sample sizes and ethnicity.

Conclusion:-

To the best of our knowledge this is the first study to evaluate the association of FVL and G20210A FII prothrombin polymorphisms with CRC in Morocco. According to the data, no significant association was observed between the FVL variant and CRC. By contrast, the G20210A FII variant was significantly associated with an increased risk of CRC development, thus suggesting that this polymorphism may be a potential risk factor for genetic susceptibility to CRC in the Moroccan population. Studies on larger populations are needed to evaluate the possible association of clotting factor gene polymorphisms and CRC risk, this may allow the discovery of new therapeutic targets for the prevention or treatment of cancer.

Competing Interests:-

The authors declare that they have no financial or nonfinancial competing interests.

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