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Frequency of the C677T Polymorphism of MTHFR, G20210A of Prothrombin and R506Q of Factor V Leiden in Type 2 Diabetics in Abidjan

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Abstract

In Africa, the prevalence of diabetes is escalating and remains a concern due to the numerous complications it causes. Vascular damage associated with diabetes leads to a prothrombotic state observed in diabetic individuals. Diabetes is a complex and multifactorial disease involving genetic components. With the aim of preventing complications and contributing to an efficient management of diabetes, we investigated genes likely to lead to a risk of thrombosis, in particular the C677T of MTHFR, G20210A of prothrombin, and R506Q of factor V Leiden in type 2 diabetics in Abidjan receiving ambulatory care. A descriptive cross-sectional study was carried out on consenting type 2 diabetic patients. Mutation detection was carried out using the PCR-RFLP method employing restriction enzymes. Hemostasis tests (fibrinogen, D-dimers, fibrin monomers, and von Willebrand factor) were performed using citrate tubes on the Stage® Star Max automated system. Plasminogen activator inhibitor was assayed by ELISA method, and biochemical parameters were determined using the COBAS C311. The study population consisted of 45 diabetic patients, 51.1% of whom presented vascular complications, mainly neuropathy. Disturbances in hemostasis parameters were observed, with 15.5% of patients showing an increase in fibrin monomers. Mutation analysis revealed an absence of factor V mutation (factor V Leiden) and of G20210A mutation of the prothrombin gene. However, 15.6% of subjects had a heterozygous C677T mutation of MTHFR, with 57% of them being anemic. The ex-

ploration of biological and genetic factors associated with thrombotic risk is of significant interest in the optimal management of African type 2 diabetics.

Keywords

Type 2 Diabetes, C677T of MTHFR, G20210A, Factor V Leiden, Thrombosis

1. Introduction

Diabetes is a complex and multifactorial disease involving both environmental and genetic components [1]. It is a chronic disease, and the number of individuals affected by it continues to rise over the years. Type 2 diabetes is the most common type [2]. In 2021, according to the International Diabetes Federation (IDF), the estimated number of people with diabetes was 537 million, resulting in 6.7 million deaths. Prevalence estimates for 2030 project 643 million affected individuals worldwide, increasing to 783 million by 2045 [3].

While diabetes was long considered a condition of affluent nations, it has now become a concern in developing countries due to the numerous complications it brings, including stroke, diabetic foot, retinopathy, nephropathy, and more [4] [5]. Diabetes complications result from both microvascular and macrovascular damage, consequences of insulin resistance and chronic hyperglycemia seen in diabetes [6]. Indeed, diabetic individuals are at high risk of thrombosis, which, in turn, contributes to cardiovascular diseases [7]. Atherothrombosis is the primary cause of morbidity and mortality in patients with diabetes mellitus [8]. It accounts for 80% of deaths and manifests as vascular and/or cardiovascular complications [7] [9] [10].

The advent of molecular biology techniques made it possible to identify genetic mutations that may predispose diabetic patients to thrombotic risk [11]. Mutations such as C677T in the methylenetetrahydrofolate reductase (MTHFR) gene, the G20210A mutation in the prothrombin gene and R506Q mutation in the factor V Leiden have been described [11] [12].

Polymorphisms in MTHFR have already been associated with type 2 diabetes and some of its complications [13] [14] [15]. A gene polymorphism results in reduced enzymatic activity of methylenetetrahydrofolate reductase, leading to decreased homocysteine metabolism and causing hyperhomocysteinemia [16]. Hyperhomocysteinemia causes dysfunction of the vascular endothelium, activates the coagulation system and inhibits the fibrinolytic system, thereby leading to a risk of thrombosis [17] [18].

The G20210A mutation in the prothrombin gene leads to a gain of function resulting in elevated thrombin levels [19] [20]. Thrombin converts fibrinogen into fibrin, promoting the formation of blood clots which leads to hyper-coagulability of the blood. The mutated factor V Leiden is less sensitive to inactivation by acti-

vated protein C, contributing to thrombotic risk as well. Taking all of the above into consideration, we aimed to conduct a preliminary.

2. Material and Methods

A descriptive cross-sectional study was conducted at the Anti-Diabetic Center of Abidjan (CADA) where patients were recruited. Biological analyses were performed at the Medical Biology Laboratory of the National Institute of Public Health (INSP) and the Molecular Biology Unit of the Center for Diagnosis and Research on AIDS and other Infectious Diseases (CeDReS) at the University Hospital Center of Treichville (CHUT) in Abidjan, Côte d'Ivoire, for the PCR-RFLP technique.

The study population consisted of randomly selected fasting type 2 diabetic patients of all genders attending consultations at CADA. Informed consent was obtained from the patients after providing them with all relevant study These patients were randomly included patients with confirmed type 2 diabetes care for at CADA. Sociodemographic, clinical, and biological data were collected using a survey form. A unique code was assigned to each patient to ensure confidentiality.

Venous blood was obtained from the elbow crease in different tubes, one with ethylenediaminetetraacetic acid (EDTA), one containing 3.2% trisodium citrate and one tube with a red cap containing a coagulation activator.

The measurement of blood glucose, total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides was performed using the Roche Cobas C311 automated analyzer from the anticoagulant-free tube (red tip). Prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, D-dimers, fibrin monomers, and von Willebrand factor were measured using citrated tubes on the Stago's Star Max automated system.

Plasminogen activator inhibitor was assayed using the ELISA method. A portion of the total blood collected in EDTA tubes was used for a complete blood count (hemogram) and the measurement of glycated hemoglobin. The second part was allotted in cryotubes and stored at −80˚C for subsequent PCR-RFLP. This technique combines amplification of specific fragments with the use of endonucleases called restriction enzymes, which recognize and cleave specific DNA sequences. The HIND III restriction enzyme was used to detect the G20210A mutation and factor V leiden. HINF 1 was used for the C677T mutation.

After thawing at room temperature, the total blood was used for genomic DNA extraction with the Qiagen QIAmp DNA Blood Mini kit. Genomic DNA extracts were amplified using two PCRs with three pairs of sense and antisense primers specific for prothrombin, MTHFR and factor V Leiden. Amplification products underwent enzymatic digestion with Hind III and Hinf I for 16 hours at 37˚C in a Marie sec bath. Enzymatic restriction products were resolved on a 1.5% agarose gel. After the enzymatic restriction, the PCR generated a band of

384 bp, 209 bp and 175 bp respectively for the G20210A, R506Q and C677T mutations.

2.1. Ethical Considerations

The study was approved by all relevant scientific authorities in the participating centers. Informed consent was collected prior to the inclusion in the study.

2.2. Data Analysis and Processing

Data processing was carried out using IBM SPSS Statistics 22 software. The results are presented in the form of tables. Quantitative variables are displayed as mean ± standard deviation and in proportion.

3. Results

3.1. Sociodemographic Characteristics of Type 2 Diabetic Patients

The study population consisted of 45 diabetic individuals with a mean age of 53 years. Females predominated, with a sex ratio of 0.4, and most of them lived in couples. The main activity carried out by diabetic individuals was self-employment (35.5%), and 26.6% of diabetics were unemployed.

3.2. Clinical Characteristics

Half of the patients had been diabetic for less than 5 years and presented glycemic imbalance. Forty percent of the patients were overweight, and complications were predominantly characterized by neuropathies. Over 70% of patients had a cardiovascular risk factor (Table 1).

3.3. Biological Parameters

On average, patients had hyperglycemia, increased fibrin monomers, antigen and Willebrand factor activity (Table 2).

All the hemostasis parameters were disturbed, except for TCa and TQ (Figure 1).

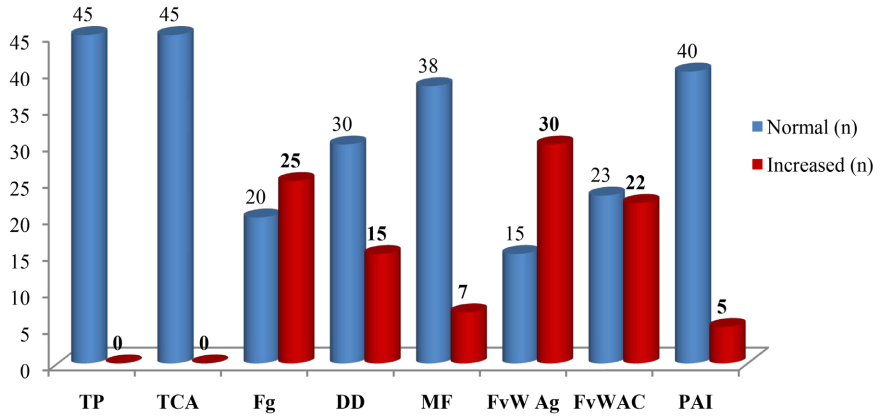

Figure 1. Variation in haemostasis parameters in the study population.

Table 1. Distribution of patients according to clinicals characteristics of patients.

	Clinical characteristics	
	Frequency (n)	**Percentage (%)**
Diabetes duration		
<5 years	23	51.1
5 - 10 years	10	22.2
>10 years	12	26.3
Blood glucose monitoring		
Imbalanced	22	49.9
Balanced	23	51.1
Total	45	100
Body Mass Index		
Normal	15	33.3
Moderate obesity	6	13.3
Severe obesity	1	2.2
Overweight	18	40.0
Unknown	5	11.1
Total	45	100
High blood pressure		
No	24	53.3
Yes	18	40.0
Unknown	3	6.7
Total	45	100
Type of complications		
Absence	22	48.9
Neuropathy	8	17.8
Nephropathy	2	4.4
Retinopathy	2	4.4
Diabetic foot	3	6.7
Stroke	3	6.7
Others	1	2.2
Unknown	4	8.9
Total	45	100
cardiovascular risk factors (FDR)		
None	10	22.2
1 FDR	16	35.6
2 FDR	18	40.0
3 FDR	2	4.4
Total	45	100

Table 2. Mean values of biological parameters.

Parameters	Mean	SD	Min	Max
Blood sugar (g/l)	1.52	0.78	0.34	3.62
HbA1C (%)	8.38	2.8	6	19
Uremia (g/l)	0.27	0.17	0.10	1.05
Creatininemia (mg/l)	9.15	4.6	4.3	27.7
Cholesterolemia (g/l)	1.96	0.57	0.98	3.43
Triglyceridemia (g/l)	1.15	0.53	0.5	2.81
HDL Cholesterol (g/l)	0.54	0.15	0.3	0.9
LDL Cholesterol (g/l)	1.19	0.53	0.4	2.6
Prothrombin ratio (%)	96	4.65	83	100
Activated Partial Thromboplastin Time (s)	33.69	4.21	26.2	51.1
Fibrinogenemia (g/l)	4.17	0.89	2.44	6.75
Fibrin monomers (ug/ml)	12.36	22.57	5	116.
D-dimers (ug/ml)	0.56	0.46	0.27	2.63
PAI (ng/ml)	14	9.89	3.51	44.3
vWF: Ag (%)	186.47	73.86	70	420
vWF: AC (%)	160	51.4	45	334

We observed an absence of the G20210 mutation in the prothrombin gene and the R506Q mutation in factor V for the entire population. The mutation C677T in the MTHFR gene was observed in 15.6% of the subjects in the heterozygous form (**Table 3**).

6/7 of patients had diabetes for less than 5 years. 4/7 of patients were overweight (**Table 4**).

All patients with a mutation exhibited erythrocyte abnormalities, with 4 out of 7 patients experiencing anemia. Additionally, 4 out of 7 patients had elevated levels of fibrinogen, von Willebrand factor antigen, and activity (**Table 5**).

4. Discussion

Diabetes and its complications represent a major public health challenge. given the growing number of diabetics and the numerous vascular complications it causes [9] [21]. Diabetic patients have an increased risk of developing thromboembolic diseases as the diabetic state contributes to their occurrence [9] [22]. Thrombotic pathologies are multifactorial and polygenic [23]. This preliminary study conducted in 45 type 2 diabetic patients sought to identify susceptibility genes for thrombosis (C677T of MTHFR. factor V Leiden. and the G20210A mutation in the prothrombin gene) in these outpatient-followed patients [24].

Table 3. Frequency of mutations in the study population.

	Frequency of mutations		
	Absence of mutation	Homozygous mutations	Heterozygous
G20210A	45 (100%)	0 (0%)	0 (0%)
R506Q	45 (100%)	0 (0%)	0 (0%)
C677T	38 (84.4%)	0 (0%)	7 (15.6%)

Table 4. Description of diabetic patients with MTHFR C677T mutation according to clinical and biochemical parameters.

Sex	Age (years)	Profession	Marital status	Diabetes duration	Complications	FDR	Obesity	Blood glucose level	HbA1C
M	70	Retired	Married	>10 years	Diabetic foot	Yes	Normal	Normal	Balanced
M	43	Worker	In couple	<5 years	None	No	Normal	Hyperglycemia	Imbalanced
F	62	Unemployed	Married	<5 years	None	Yes	Overweight	Normal	Balanced
F	51	Self-employed	Widow	<5 years	None	No	Overweight	Normal	Balanced
F	46	Other	Married	<5 years	Nephropathy	Yes	Overweight	Normal	Balanced
F	40	Unemployed	Married	<5 years	Neuropathy	No	Overweight	Normal	Balanced
F	50	Self-employed	Single	<5 years	None	No	Normal	Hyperglycemia	Imbalanced

Table 5. Distribution of diabetics with the MTHFR C677T mutation according to erythrocyte and haemostasis parameters.

Sex	Erythrocyte parameters	Fg (g/l)	DD (ug/ml)	MF (ug/ml)	PAI (ng/ml)	vWF: Ag	vWF: Ac
M	NMA*	Increased	Normal	Normal	Normal	Increased	Increased
M	Hypochromia	Increased	Normal	Normal	Normal	Increased	Increased
F	Hypochromia	Normal	Normal	Normal	Increased	Increased	Increased
F	A* Hypochromia	Normal	Normal	Increased	Normal	Normal	Normal
F	NMA*	Increased	Increased	Increased	Normal	Increased	Increased
F	NMA*	Increased	Increased	Increased	Normal	Normal	Normal
F	AHM*	Normal	Increased	Normal	Normal	Normal	Normal

*A: Anemia, NMA: Normochromic Microcytic Anemia, MHA: Microcytic Hypochromic Anemia.

The mean age of this study population was 53 years with a predominance of females. These observations align with similar African studies carried out in Senegal and Algeria [24] [25]. Age is considered an important risk factor for thrombosis and its incidence is higher in the elderly than in the young, which may be explained by reduced mobility, decreased muscle tone, aging veins and above all the acquisition of risk-increasing diseases such as type 2 diabetes [26] [27]. In this study, more than half the diabetic patients had a diabetes duration of less than 5 years and presented complications. Neuropathy was predominant. Diabetes duration exceeding 5 years increases the likelihood of complications

[28]. In our countries, diabetes screening is often delayed as patients seek consultation when clinical signs are already evident, which reflect a poor estimation of this duration. In Africa, several authors have described the prevalence of microvascular complications as observed in this study [4] [29]. Cardiovascular risk factors, including arterial hypertension and obesity, were found in 40% and 15.5%, respectively. Nearly 40% of patients were overweight. These parameters vary in frequency in several African studies, although they are frequently encountered risk factors in diabetes [29] [30]. Chronic hyperglycemia in diabetes is responsible for vascular damage that can lead to platelet activation and initiate coagulation. Additionally, glycemic imbalance also exposes the patient to a thrombotic risk [31] [32]. The control of glycated hemoglobin, a predictive marker of diabetic complications, is closely linked to the prevention of micro- and macrovascular complications in diabetes [31]. This glycemic imbalance was observed in our study population with an HbA1C level of 8.38%. An HbA1C value higher than 7% is known as a factor for thrombotic events in diabetes [33]. The study of hemostasis parameters has revealed a disturbance in these parameters, indicating coagulation activation in diabetic patients. Fibrin monomers, early markers of coagulation activation, found at an early stage of thrombotic disease, were increased in 15.5% of patients [33]. This observation underscores the importance of early assessment of thrombotic risk to ensure better management of complications. Fibrinogen and von Willebrand factor were elevated in more than half of the patients. Chronic inflammation can be both the cause and consequence of endothelial dysfunction, promoting coagulation activation. This increase in pro-inflammatory proteins especially fibrinogen and Willebrand factor has already been described by some authors [34] [35]. The Willebrand factor is an important indicator of endothelial dysfunction. All these findings support coagulation activation and hence to a risk of thrombosis that contributes to vascular complications in diabetic patients [36].

The search for genetic markers revealed an absence of factor V leiden and the G20210A mutation of the prothrombin gene in the study population. The mutated allele of the prothrombin gene was not found in our patients—these results are similar to previous studies in Africa [37] [38] but contrary to studies carried out in Algeria which found this mutated gene in their study population [39] [40]. We noted an absence of the factor V mutation in our type 2 diabetic patients. The absence of this mutation should prompt discussion of the presence of other factor V mutations such as the Cambridge or Hong Kong mutations and their possible involvement in thrombosis occurrence [41] [42]. Factor V mutations account for around 1% of the black American and African people, while the G20210A mutation in the prothrombin gene is rare in the African population [43] [44]. Studies on the frequency of these mutations in our population in general and in the diabetic population in particular will enable us to corroborate or identify specificities in our diabetic patients. The heterozygous C677T mutation of the MTHFR gene was found in 7 patients (15.5%). This observation is

similar to a Tunisian study which found this mutation in diabetic patients. The heterozygous C677T mutation of the MTHFR gene was found in 7 patients (15.5%). This observation is similar to a Tunisian study that found this mutation in diabetic patients. This prevalence was positively associated with hyperhomocysteinemia. This association between hyperhomocysteinemia and heterozygous genotype is more pronounced in diabetics with nephropathy [45] [46]. Analysis of the clinical and biological parameters in the 7 diabetic patients with the C677T mutation shows that 6 out of 7 of these patients have had diabetes for less than 5 years. 4 out of 7 are overweight and 3/7 experiencing microvascular complication. Early detection of this mutation in a diabetic population will contribute to a better understanding of the pathophysiological mechanisms of diabetes. All 7 patients had normochromic normocytic anemia (3/7) and hypochromic anemia (4/7). In Pandya's study, patients with iron-deficiency anemia and the C677T genetic marker for thrombosis had lower hemoglobin levels and hematimetric constants compared to those without the genetic marker [47]. This avenue should be explored in order to determine whether anemia, and in particular iron-deficiency anemia could be used to search for a C677T mutation in diabetics. The association between the C677T polymorphism and the risk of developing type 2 diabetes, as well as with complications, has already been reported in the literature [25] [48], although this assertion is controversial as some studies conducted in Germany and Turkey found no association [49] [50]. It seems appropriate to investigate this mutation in a larger sample in order to identify specificities in the Black population in general and particularly in the Ivorian population. This can be achieved by determining the frequency of these mutations and exploring a potential link between gene polymorphisms and the occurrence of complications in diabetic individuals.

5. Conclusion

This preliminary study conducted among outpatient type 2 diabetic individuals seen on an outpatient basis to investigate susceptibility genes for thrombosis revealed the presence of the C677T mutation in 15.5% of patients alongside disturbances in hemostasis parameters. The exploration of biological and genetic factors related to thrombotic risk holds significant interest in the knowledge and efficient management of black African type 2 diabetic patients.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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