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Precision medicine as a predictive factor for risk of hospitalization of recurrent ischemic stroke patients treated with low dose aspirin. A pilot study

Eman AlHussain A. Gawad [a], Mevidette El Madani [b], Hanan Mostafa [c] and Radwa Marwan Abdel halim [a]

[a]Chemical Pathology Department, Cairo University, Cairo, Egypt; [b]Pharmacology Department, National Research Centre, Giza, Egypt; [c]Department of Anesthesia, surgical intensive care medicine and pain, Cairo University, Egypt

ABSTRACT

Recurrent ischemic stroke risk after transient ischemic attack was underestimated by most of epidemiological studies due to the widespread use of very different and sometimes restrictive definitions for recurrent stroke instead of a standard definition, making it difficult to assess the benefit of early prevention of recurrence. Knowing that early intervention by treatment with aspirin reduced fatal recurrent stroke and proved to be cost effective by reducing hospital admission, it was worthwhile studying the pharmacogenetics association as well as biological risk factors of aspirin intolerance for patients with recurrent ischemic stroke. Accordingly, our primary outcome was to investigate the association of *CYP2C9*3* (Rs1057910) polymorphism as well as other factors such as diabetes, hypertension and smoking with aspirin intolerance for these patients. The secondary outcome was to study the correlation between TNF-alpha as an inflammatory mediator and the prognosis of ischemic stroke for these patients. Our results showed that although smoking and heterozygous A/C of Rs1057910 still have a higher prevalence however statistically insignificant risk for ischemic stroke recurrence compared with other risk factors; OR1.69 and 1.9, 95%CI (0.471–1.695) and (0.321–11.257) respectively. Also, our results suggest that there is a borderline statistically significant correlation between recurrent stroke prognosis and TNF alpha ($p = 0.043$). Conclusion: This study failed to detect association of Rs1057910 variant of *CYP2C9* gene with preventive aspirin for patients with ischemic stroke. However, a somehow significant association was detected between ischemic stroke prognosis and TNF- α as an inflammatory marker. Further research investigations on larger sample size population are encouraged.

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1. Introduction

Recurrent stroke events represent a higher risk of disability and mortality than first strokes after transient ischemic attacks (TIA) [1]. However, very few studies have been initiated to address the question about the recurrent stroke absolute risk after TIA. Therefore, we are lacking in reliable data that would accurately provide an answer to this question. It is reported in many studies that the recurrent stroke risk is reaching 8–10% during the first 7 days after a TIA or minor stroke [2,3]. No precise estimates of the effectiveness, or costs for urgent assessment neither preventive treatment is available for such patients.

Rothwelland his clinical team reached the conclusion that studying the effect of urgent treatment of transient ischemic attacks on early recurrent stroke to both individual and public health is crucial [3]. The EXPRESS study highlighted the benefits of early prevention of recurrent stroke by proving that rapid assessment of TIA and minor stroke in combination with rapid interference for acute treatment resulted in marked reduction for the risk of recurrent stroke by nearly 80%. Occurrence of fatal or disabling recurrent strokes decreased, hospital admission costs, and disability levels at 6-month follow-up [4]. Many recurrent stroke prevention treatment protocols have been suggested, among which aspirin has proven to be of great benefit in reduction of of recurrent ischemic stroke's relative risk [4]. The outcome of grouped data from the Chinese Acute Stroke Trial and the International Stroke Trial was a 30% reduction of stroke recurrence at 14 days ($p < 0 \cdot 00001$) after treatment with aspirin [5].

The study of pharmacogenomics, as well as relevant polymorphisms of aspirin-related genes and their association with aspirin metabolism, pharmacokinetics, and intolerance, is critical. Knowledge of the genetic factors underlying variability in aspirin metabolism could provide additional insight into the cause of aspirin intolerance. It was previously given that polymorphisms in the genes coding for three specific enzymes, namely *UGT1A6, ACSM2B, CYP2C9,* are estimated to be involved in aspirin metabolism and therefore considered as major pharmacogenomics targets in aspirin intolerance [6]. Thus,

CONTACT Eman AlHussain A. Gawad emanalhussain@cu.edu.eg Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Al-Saraya Street, AlManial, Cairo, Egypt, 11956

major changes to the human CYP2C9 gene include the following: CYP2C9*2, which causes an amino acid substitution at position 144, and CYP2C9*3, which causes an amino acid exchange at position 359, are both linked to decreased drug metabolism [6,7]. CYP2C9*5 causes an amino acid substitution in position 360, CYP2C9*6 causes an amino acid substitution in position 273, and CYP2C9*11 causes an amino acid substitution in position 335.

Several examples of impaired drug metabolism in vivo due to variant alleles for CYP2C9*2 or CYP2C9*3 have been provided, with drugs such as warfarin, several NSAIDs, hypoglycemics and angiotensin antagonists [*8,9]. It should be noted that the frequencies of common CYP2C9 single nucleotide polymorphism vary greatly across ethnic groups (SNPs) [10,11]. This should be taken into consideration while studying the effect of *CYP2C9* enzyme activity and its relationship to aspirin intolerance as a great variation might be observed depending on the study population.

The current research might open new perspectives on preventive therapy of ischemic stroke recurrence by applying the concept of precision medicine. This concept consists of studying the genetic profile of a specific population with the aim of tailoring a specific treatment targeting their genetic polymorphism. Our study was conducted on Egyptian population with the aim of studying the genotypic association between **Rs1057910** variant of (*CYP2C9*3*) gene to the effect of aspirin and recurrence of ischemic stroke patients who were given aspirin immediately after stroke diagnosis. We hypothesized that this would help in identification among the patients most likely to benefit from aspirin as a preventive treatment to recurrence of ischemic strokes in this specific population and also giving the chance to develop targeted therapy to recurrent patients resistant to aspirin based on their genetic profile.

2. Subjects and Methods

This cross-sectional pilot study included 54 ischemic stroke patients hospitalized at Kasr Al Aini Hospital in surgical intensive care and stroke unit from January 2019 to January 2021. All patients in this study were given low-dose aspirin as prophylaxis to ischemic stroke recurrence. The data analysis used in this study were based on 12 weeks' low-dose aspirin administration to these patients starting from the occurrence of ischemic stroke and represented in this paper as descriptive statistics.

3. Patients

Subjects were divided into two groups:

Group A included: 14 patients with recurrent ischemic stroke documented clinically and radiologically with CT scan ranging in age from 52 to 75 years.

Group B included: 40 patients with non-recurrent ischemic stroke excluded clinically and radiologically with CT scan ranging in age from 45 to 80 years.

Inclusion criteria: confirmed cases of Ischemic stroke by imaging either recurrent identified by CT scans or non-recurrent treated with low-dose aspirin immediately after diagnosis. Exclusion criteria were patients who had cardiac problems.

Groups were subjected to the following: (i) full history taking, including: present history symptoms and signs, medication history and past history of previous stroke, diabetes history, hypertension history, and history of relapse. (ii) blood pressure was measured twice – the diagnosis of ischemic stroke was made in accordance with the American Heart Association Guidelines (AHA), 2009 [12]; (iii) Early CT and MRI examination for confirmation of ischemic stroke and subtypes large atherosclerotic artery vs small atherosclerotic artery (LAA vs SAA); (iv) laboratory investigations: (a) Standard testing, such as a full lipid profile utilising a Beckman Coulter AU680 automated chemistry analyzer (Beckman Coulter, Inc., Brea, CA, USA); (b) Immunological tests, such as TNF-alpha carried out using Glory Science Co., Ltd.; (c) Molecular tests: utilizing the Applied Biosystems Step oneTM Real Time polymerase chain reaction equipment, an optimised protocol of the allelic discrimination genotyping assay for the Rs1057910 was developed (Applied Biosystems, Foster City, CA, USA).

4. Sample Collection

Each participant provided 5 mL of fasting venous blood which was divided into two vacutainer tubes after a consent was obtained from patient if fully conscious or next of kin available and legible for consenting as first-degree relative, The sample was divided as follows:

3 mL of blood in a basic, sterile, dry vacutainer. The samples were centrifuged at 3000 g for 10 min after being allowed to clot at room temperature. The total cholesterol (TC), high-density lipoproteins (HDL-c), low-density lipoproteins (LDL-c) and triglycerides were all measured in the initial aliquot of serum, which was then immediately frozen at −20 degrees Celsius for the TNF- test.

2 mL of blood in a sterile violet-topped ethylene diamine tetra acetate (EDTA) −20°C in a microcentrifuge tube for genotyping of **Rs1057910** variant by RT-PCR.

5. DNA extraction and genotyping

Vacuum EDTA tubes were used to collect blood samples. Using a QIAamp DNA blood micro kit, genomic DNA was extracted from whole blood (Qiagen, Germany).

The **Rs1057910** variant of *CYP2C9* gene was genotyped across all subjects and sample sets were run using a Step-one Real-Time Polymerase Chain Reaction System and the TaqMan Allelic Discrimination Assay Kit (Applied Biosystems, Foster City, CA, USA; probe ID C 27104892 10).

6. Parameters

Three parameters were evaluated regarding ischemic stroke recurrence: Ischemic stroke prognosis, **Rs1057910** (A/C) heterozygous gene, TNF-α. The frequency of *variant allele A and C* (**Rs1057910**) association with ischemic stroke recurrence, artery subtypes (LAA/ SAA); according to the stroke severity classification and Trial of Org 10,172 in Acute Stroke Treatment (TOAST) prognosis was also evaluated. NIHSS score is defined as the sum of 15 individually evaluated elements, and ranges from 0 to 42. Stroke severity may be categorized as follows: **no stroke symptoms, 0; minor stroke, 1–4; moderate stroke, 5–15; moderate to severe stroke, 16–20; and severe stroke, 21–42**. The TOAST classification denotes five subtypes of ischemic stroke: (1) large-artery atherosclerosis, (2) cardio-embolism, (3) small-vessel occlusion, (4) stroke of other determined etiology and (5) stroke of undetermined etiology.

7. Theory

Precision medicine could be used as a predictive tool to help healthcare professionals to decide on which patients are most likely to benefit from aspirin given as preventive therapy for ischemic stroke recurrence. This theory was applied and tested to the current study by evaluating the association between one single nucleotide polymorphism for *CYP2C9,* namely rs1057910 (*CYP2C9*3*), and the effect of recurrence in ischemic stroke patients who were given aspirin as preventive measure against stroke recurrence immediately after diagnosis. *CYP2C9*3* is proved to be one of the major variants affecting the human *CYP2C9* gene involved in aspirin metabolism.

8. Calculation/statistical analysis

The statistical package for the social sciences (SPSS) version 26 was used to code and enter the data (IBM Corp., Armonk, NY, USA). For quantitative variables, mean and standard deviation were used to describe the data, and for categorical variables, frequencies (the number of cases) and relative frequencies (percentages) were used. Unpaired t tests were employed to compare groups for quantitative variables with normally distributed distributions, whereas non-parametric Mann-Whitney tests were used for those with non-normally distributed distributions [13]. An analysis using the Chi square (2) test was done to compare categorical data. When the anticipated frequency is less than 5, the exact test was utilised in its place [14].

The Spearman correlation coefficient was used to determine correlations between quantitative variables [15]. To find the optimal TNF-alpha cutoff value for detecting a heterozygous allele, an area under curve analysis was performed before building the receiver operating characteristic curve analysis (ROC) curve. To find independent recurring predictors, logistic regression was used [16]. Statistics were considered significant for P-values under 0.05.

9. Results

9.1. Association of preventive aspirin to the recurrent ischemic stroke prognosis and routine laboratory investigations

National Institutes of Health Stroke (NIHSS) scaling where NIHSS scale more than 16 is considered as bad prognosis. Forty-two (42) patients were NIHSS less than 16 and they were classified as mild and moderate. The other 12 patients showed NIHSS more than 16 and they were classified as severe as shown in Table 1. The early benefit of aspirin administration in our study was demonstrated by a statistically significant difference in the prognosis of the recurrent ischemic stroke patients compared to non-recurrent patients (mean 16.4 vs 6.41 respectively; p = 0.021) as shown in Table 2. On the other hand, lipid profile such as high-density lipoprotein (HDL-c), cholesterol (CH), triglycerides (TG), low-density lipoprotein (LDL-c) and inflammatory mediator (TNF-alpha) showed a non-significant statistical difference between recurrent and non-recurrent ischemic stroke patients. The recurrence of ischemic stroke was independent of all laboratory investigations as shown in Table 2 and Figure 1 (Appendix A-Figure 1).

Table 1. The severity of prognosis after 12 weeks of aspirin administration.

		Number of patients (n)	Percentage %
Severity	Mild to moderate	42	77.8%
	severe	12	22.2%

Table 2. Routine laboratory investigations of studied patients.

	Recurrent				
	YES		NO		P value
	Mean	Standard Deviation	Mean	Standard Deviation	
HDL-c (mg/dl)	38.07	9.88	33.88	13.41	0.289
T. Chol. (mg/dl)	197.50	35.37	203.83	41.08	0.610
TG (mg/dl)	180.29	83.49	154.32	60.80	0.390
NIHSS	16.14	7.07	12.30	6.41	0.021*
LDL-c (mg/dl)	123.66	35.03	140.75	40.84	0.169
TNF-alpha (pg/ml)	63.57	20.68	54.58	30.40	0.418

*Statistically significant value (p-value<0.05); Data are presented as mean and standard deviation.
[1]HDL-c, high-density lipoprotein; CH, cholesterol; TG, triglyceride; NIHSS: National Institutes of Health Stroke scaling; LDL-c, low-density lipoprotein; TNF-alpha, Tumor necrosis factor alpha.

9.2. Association of preventive aspirin to Rs1057910 genotypes and other predictor risk factors of ischemic stroke recurrence

The data of this study showed non-statistically significant difference in recurrent ischemic stroke patients with heterozygous variation A/C genotypes versus recurrent ischemic stroke patients with wild AA genotypes. Also, the prevalence of diabetes, hypertension and smoking did not reach statistically significant difference in recurrent ischemic stroke patients in comparison to non-recurrent ischemic stroke patients as described in Table 3.

An adjusted odds ratio (AOR) for risk of ischemic stroke recurrence using multivariate logistic regression analysis with the conventional risk factors included into consideration hypertension, smoking, diabetes, age, LDL-c, total cholesterol and heterozygous genotype. Smoking and heterozygous A/C of **Rs1057910 prevalence** still have a higher, but statistically insignificant, risk for ischemic stroke compared with other risk factors; OR 1.69 and 1.9, 95% CI (0.471–1.695) and (0.321–11.257) respectively as shown in Table 4.

10. Association of heterozygous gene with severity of stoke and with tumor necrosis factor alpha (TNF- α)

There was no statistically significant difference regarding stroke prognosis in patients with heterozygous A/C genotypes (mean = 12.30, SD = 5.810) versus patients with AA genotypes (mean = 13.52, SD = 6.97) (p value = 0.695). Same as TNF-alpha, as shown in Table 5, patients with heterozygous A/C genotypes (mean = 62.40, SD = 28.52) versus patients with AA genotypes (mean = 55.66, SD = 28.42) (p value = 0.593).

10.1. The frequency of variant allele A and C (Rs1057910) association with ischemic stroke recurrence, artery subtypes and severity of stroke prognosis

Patients with non-recurrent ischemic stroke are significantly more prevalent than those with recurrent ischemic stroke which is estimated to be 40 (74.1%) versus 14 (25.9%) respectively. There were 3/28 (30%) patients with allele C and 25/28 (25.5%) patients with allele **A** in the recurrent group. On the other hand, there were 7/80 (70%) patients with allele C and 73/80 (74.5%) patients with allele **A** in the non- recurrent group. Our results demonstrated that there was no association of allele C and allele A with ischemic stroke recurrence (p = 0.7170). Our findings also confirmed that there was no association of allele c and allele A with artery subtypes (LAA and SAA) (p = 0.520) and severity of ischemic stroke prognosis (p = 0.4530). The data are represented in Table 6.

11. Relation of tumor necrosis factor alpha (TNF- α) to stroke prognosis

The results of our findings as shown in Table 7 suggest that there was a borderline statistically significant correlation between recurrent stroke prognosis and TNF – alpha (p = 0.043)

12. Receiver operating characteristic curve analysis (ROC) analysis for the sensitivity of TNF-α^7 to discriminate the heterozygous allele from the wild one

Receiver operating characteristic curve analysis: ROC curve analysis was performed to evaluate the usefulness of TNF-α^7 as potential markers to discriminate between ischemic stroke groups with A/C genotype (heterozygous allele) versus mutant genotypes (AA).

Table 3. Association of prophylactic aspirin to *Rs1057910* genotype and routine clinical investigations.

		Recurrent				
		YES		NO		
		Number of patients (n)	Row N %	Number of patients (n)	Row N %	P value
DM	YES	4	22.2%	14	77.8%	0.751
	NO	10	27.8%	26	72.2%	
HTN	YES	12	34.3%	23	65.7%	0.102
	NO	2	10.5%	17	89.5%	
Smoking	YES	6	31.6%	13	68.4%	0.528
	NO	8	22.9%	27	77.1%	
Rs1057910	A/C	3	30.0%	7	70.0%	0.708
	AA	11	25.0%	33	75.0%	

[2]DM, Diabetes Mellitus; HTN, Hypertension.

Table 4.: Multivariate logistic regression analysis with adjustment of the traditional risk factors hypertension, diabetes mellitus, low-density lipoprotein-c.

				95% C.I.	
		P value	AAOR	Lower	Upper
Stoke recurrence	Age	0.947	1.002	0.932	1.078
	HDL-c (mg/dl)	0.700	1.012	0.953	1.074
	CH (mg/dl)	0.351	1.024	0.974	1.077
	TG (mg/dl)	0.804	1.002	0.989	1.014
	NIHSS	0.119	1.080	0.980	1.189
	LDL-c (mg/dl)	0.189	0.966	0.917	1.017
	TNF- α (pg/ml)	0.161	1.021	0.992	1.052
	DM	0.748	0.765	0.149	3.921
	Smoking	0.471	1.695	0.403	7.125
	Rs1057910 (A/C)	0.479	1.901	0.321	11.257

[3]HDL-c, high-density lipoprotein; CH, cholesterol; TG, triglyceride; NIHSS: National Institutes of Health Stroke scaling; LDL-c, low-density lipoprotein; TNF-alpha, Tumor necrosis factor alpha. DM, Diabetes mellitus.

ROC area under the curve for TNF-alpha in ischemic stroke showed a p value of 0.594 as shown in Table 8 and Figure 2 (Appendix B- Figure 2).

13. Discussion

The current study evaluates the significance of genetic variations in enzymes involved in metabolism, aspirin effect, and common polymorphisms related to preventive aspirin against ischemic stroke patients' recurrence, and it raises hypotheses on genetic factors related to altered response to aspirin that need further investigation. The importance of studying genetic polymorphism that might be associated with recurrent ischemic stroke patients treated with aspirin has been addressed in our work by evaluating all patients (recurrent versus non-recurrent) administered small dose of aspirin. The benefit of studying such polymorphism in these patients is to determine the protective role of CYP2C9*3 gene expressed in recurrent ischemic stroke patients resistant to aspirin and to detect the correlation between the occurrence of recurrence and the expression of CYP2C9*3 gene polymorphism in these specific group of patients.

In the present study, we found significant decreases in the early risks of acute stroke with aspirin administration for 12-week cut-off time analysis. This was presented by the high percentage of non-recurrent ischemic stroke patients compared to recurrent ischemic stroke patients which is estimated to be (74.1%) versus (25.9%) respectively. Additionally, we discovered that a substantial percentage of aspirin's early effect was attributable to a decrease in the severity of early non-recurrent ischemic stroke. To further elaborate about the reason behind better prognosis of non-recurrent ischemic stroke in patients administered low-dose aspirin, we investigated the association of *CYP2C9 variant allele* to the effect of aspirin prophylaxis. In addition to nonspecific esterase, it has

Table 5. Association of Rs1057910 genotypes to stroke prognosis and inflammatory mediator (TNF- α).

	rs1057910				
	A/C		AA		
	Mean	Standard Deviation	Mean	Standard Deviation	P value
NIHSS	12.30	5.81	13.52	6.97	0.695
TNF-alpha	62.40	28.52	55.66	28.42	0.593

[4]NIHSS: National Institutes of Health Stroke scaling; TNF-alpha, Tumor necrosis factor alpha.

Table 6. The frequency of Rs1057910 alleles in relation to stroke recurrence, artery subtypes and severity of prognosis.

		Rs1057910 alleles				
		allele C		allele A		
		Number of patients (n)	Column N %	Number of patients (n)	Column N %	P value
Recurrent	YES	3	30.0%	25	25.5%	0.717
	NO	7	70.0%	73	74.5%	
artery groups	SAA	5	50.0%	37	39.4%	0.520
	LAA	5	50.0%	57	60.6%	
severity	mild to moderate	9	90.0%	75	76.5%	0.453
	severe	1	10.0%	23	23.5%	

[5]SAA, small atherosclerotic artery; LAA, large atherosclerotic artery.

previously been shown that three important enzymes, namely UGT1A6, ACSM2B and CYP2C9, play a significant role in the metabolism of aspirin [17–19**]. Aspirin bio disposition and, consequently, aspirin intolerance are believed to be affected by polymorphisms in the genes encoding for these enzymes. For instance, South-European Caucasians have a frequency of about 15% for the most frequent variation allele CYP2C9, while North-European Caucasians have a frequency of about 7%, and other ethnicities have significantly lower frequencies. For CYP2C9, our investigation focused on the single nucleotide polymorphism Rs1057910 (CYP2C9*3).

The results of the current study showed that smoking and heterozygous A/C of **Rs1057910** prevalence still have a higher, but statistically insignificant, risk for ischemic stroke recurrence compared with other risk factors; OR 1.69 and 1.9, 95%CI (0.471–1.695) and (0.321–11.257) respectively. We did not identify any of the two screened genotypes for this SNP; heterozygous A/C genotypes or wild AA genotype; as a relevant risk factor for patients taking aspirin early after the ischemic stroke for a period of 12 weeks. This finding suggests that CYP2C9 polymorphism is not related to prophylactic aspirin in prevention of recurrent stroke episodes.

Our results were consistent with Shiotani et al.'s (2009) analysis of the impact of various SNPs, including two SNPs for CYP2C9, designated as Rs1799853 and Rs1057910, in the risk to develop gastrointestinal bleeding with low-dose aspirin. Shiotani et al.'s study involved polymorphisms of aspirin-related genes in aspirin intolerance. Their results demonstrated that any of the SNPs that were stated earlier was a significant risk factor for gastrointestinal haemorrhage [20].

We further investigated the frequency of two **Rs1057910** alleles, allele A and allele C, on prophylactic aspirin and we found no association of allele C or allele A with prophylactic aspirin and severity of ischemic stroke prognosis. In contrast to the present findings, the odds ratio for the risk of gastrointestinal bleeding in this subgroup of patients was reported to be 1.73 for carriers of CYP2C9 variant alleles in the study by Blanco et al. (2006), which comprised 63 patients and 91 control individuals treated with aspirin. Thus, despite CYP2C9's small role in the metabolism of aspirin, it may be deduced that CYP2C9 metabolism dysfunction raises the possibility of experiencing gastrointestinal side effects from aspirin [21].

Our research supports the expression of TNF- serum levels in patients with acute recurrent stroke. This result was in line with experimental data showing that TNF-mRNA is expressed early before cytokine production [22]. This could be explained by the expression of IL-1, IL-6 and tumour necrosis factor (TNF) by platelets when monocytes are co-incubated [23,24]. TNF-alpha has been linked to stroke in a number of ways, including the start and spread of the inflammatory process [25], cell apoptosis in ischemia injury [26] and CNS reperfusion injury [27]. Furthermore, it has been demonstrated that stroke patients who have high plasma levels of IL-6, TNF-, or soluble VCAM-1 – a leukocyte adhesion molecule expressed by activated endothelium – may be more susceptible to having another stroke [28,29]. Patients with ischemic stroke have also been found to

Table 7. Correlation between stroke prognosis and tumor necrosis factor- α.

		TNF- α
NIHSS	Correlation Coefficient	0.277
	P value	0.043*
	N	54

[6]NIHSS: National Institutes of Health Stroke scaling; TNF-α, Tumor necrosis factor alpha

Table 8. Area under the ROC Curve for TNF-alpha in ischemic stroke.

			95% Confidence Interval	
	Area Under the ROC Curve	P value	Lower Bound	Upper Bound
TNF- α[7]	0.555	0.594	0.354	0.755

[7]TNF- α, Tumor necrosis factor alpha

have higher levels of the eicosanoids thromboxane A2 and MMP-9, which are indicators of platelet or mononuclear cyclooxygenase activity.

These findings raised the question about whether inflammatory process is implicated in protective aspirin to ischemic stroke prognosis or not. The effects of single or dual antiplatelet therapy (aspirin, clopidogrel and dipyridamole) and piracetam medication on TNF- and IL-8 productions were initially studied by Al-Bahrani et al. in 2007. They unexpectedly discovered that the generation of TNF- and IL-8 was inhibited by these antiplatelet medications [30]. According to these data, Yang et al. (2004) showed through their experiment that aspirin outperformed other medications in lowering TNF- and IL-8 emissions. These decreases may have happened as a result of aspirin's activity as an anti-inflammatory mediator [31]. As a result, aspirin usage could be expanded to include managing all vascular event illnesses as well as stroke.

Our findings have implications for the acute management of patients with mild strokes who have recurrent ischemic stroke. First of all, they support recommendations for quick patient assessment since they are consistent with results from other non-randomized studies regarding the influence of urgent therapy on the early risk of recurrent stroke [4,32,33]. Second, they argue that all patients should be suitable candidates regardless of any genetic predisposition and that aspirin was mostly responsible for the benefits of immediate treatment. Third, they demonstrated that aspirin lessened the severity of an early recurrent stroke in patients who hadn't been taking an anticoagulant.

Our findings also affect how the general population is educated. First, it is crucial that patients with mild strokes receive acute care since evidence suggests that prompt treatment after a minor ischemic stroke lowers the risk of recurrence and improves patient prognosis. As a result, it is important to limit patient delays in seeking medical assistance. Second, given that aspirin is widely available in households, thought should be given to encouraging self-administration as soon as temporary neurological symptoms following a stroke, similar to how it is advised for persons with acute chest pain.

14. Conclusion

A significant association was detected between ischemic stroke prognosis and TNF- α as an inflammatory marker; however, this pharmacogenetic study wasn't able to demonstrate an association of **Rs1057910** variant of *CYP2C9* gene with aspirin prophylaxis for patients with ischemic stroke. However, further studies on a larger sample size are needed for better genetic interpretation.

List of abbreviations

TIA	Transient ischemic attacks
SNP	Single Nucleotide Polymorphism
CT	Computed Tomography
MRI	Magnetic Resonance Imaging
AHA	American Heart Association Guidelines
TNF–alpha	tumor necrosis factor-alpha
LAA	Large atherosclerotic artery
SAA	Small atherosclerotic artery
TOAST	Trial of Org 10,172 in Acute Stroke Treatment
RT-PCR	Real Time polymerase chain reaction system
SPSS	Statistical Package for the Social Sciences
NIHSS	National Institutes of Health Stroke scaling
ROC	Receiver operating characteristic curve analysis
HDL	High-density lipoprotein
CH	Cholesterol
TG	Triglycerides
LDL	Low-density lipoprotein
HTN	Hypertension
DM	diabetes mellitus.

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Disclosure statement

The writers say they have no conflicting agendas.

ORCID

Eman AlHussain A. Gawad http://orcid.org/0000-0003-4343-0820
Mevidette El Madani http://orcid.org/0000-0002-2012-0929
Hanan Mostafa http://orcid.org/0000-0003-4638-417X
Radwa Marwan Abdel halim http://orcid.org/0000-0002-8611-0424

Availability of data

Participants in the study were given the assurance that raw data would be kept private and wouldn't be disclosed because the information gathered in this study was sensitive.

Data not accessible; sensitive information was used.

Ethics statement

The Director General of Health Services of Kasr AL Aini hospital approved the procedure for collecting samples from ischemic stroke patients, processing the samples, transporting the samples, and extracting the RNA from the samples.

Informed consent

Prior to collecting samples, each participant gave their written consent. The local Ethics Committee of the Clinical and Chemical Pathology Department of Cairo University authorised the project. (number: I-350316/May 2016).

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Author contributions

Hanan Mostafa, an anaesthesiologist subspecialized in surgical critical care and part of intensive care team in Kasr AL Ainy, helped in patient selection and data collection. Mevidette Adel ELMadani conceived and designed the study, performed statistical analysis, interpreted results and drafted the manuscript. Eman ALHussain A.Gawad curated the data and performed DNA extraction and genotyping analysis. Radwa Marwan Abdel Halim recruited patients, performed sample collection and reviewed the drafted manuscript.

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Appendix A

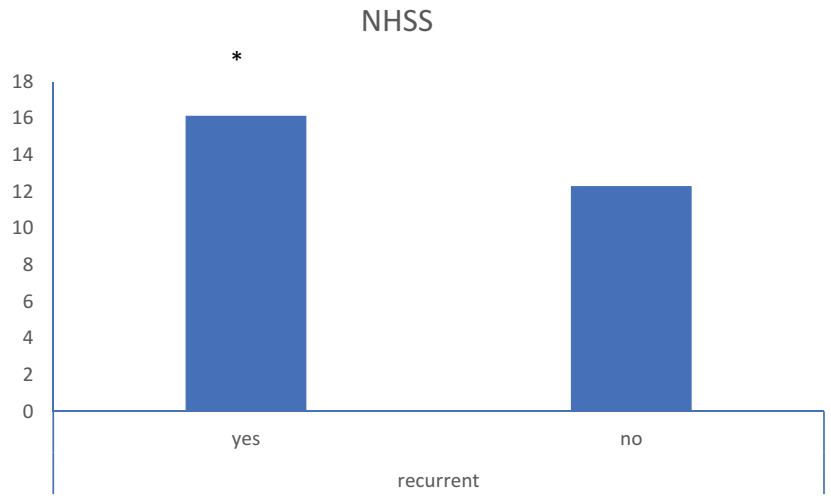

Figure 1. Bar chart representing the effect of the prognosis on ischemic stroke recurrence and non-recurrence.

Appendix B

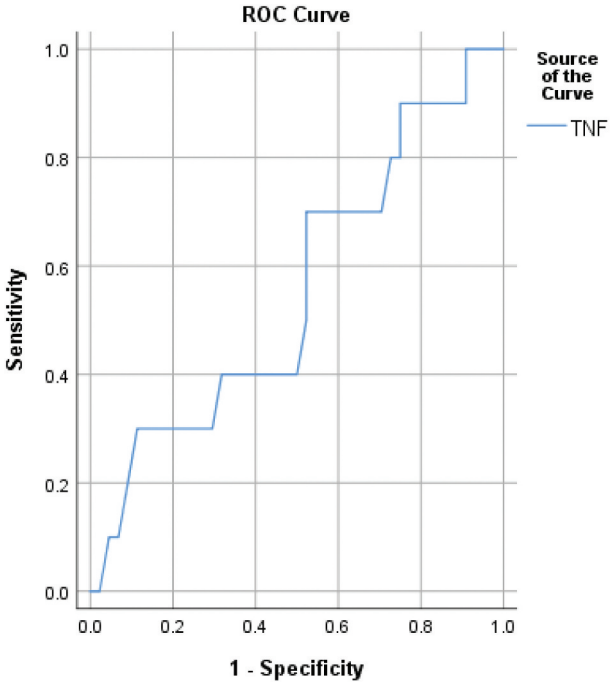

Figure 2. Receiver operating characteristic curve for TNF-alpha in ischemic stroke.