Assessing the level of infectious and inflammatory factors in acute ischemic stroke in diabetic and non-diabetic patients

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ABSTRACT

Introduction. Stroke is one of the principal leading causes of death globally. In 2005, stroke cause approximate 5.7 million death, 87% of deaths was in low and middle-income countries. Recently period, collecting evidence indicated that inflammation and atherosclerosis play important roles in stroke evolution.

Material and method. In this prospective longitudinal observational study including 340 patients with acute ischemic stroke with / without diabetes mellitus we analyzed as main criteria infectious factors, inflammatory factors and biochemical factors. The patients were divided into 2 groups: 101 diabetic patients (study group) and 239 non-diabetic patients (control group), were we analyzed as main criteria infectious factors (cytomegalovirus IgG plasma level, cytomegalovirus IgM plasma level, *Helicobacter pylori* IgG plasma level), inflammatory factors (leucocyte, C-reactive protein) and biochemical (plasma level of fibrinogen).

Results. Cytomegalovirus-IgG levels are lower in diabetic versus non-diabetic patients with an arithmetic mean of 1170 AU/ml (95% CI 862.4169 to 1477.8326) versus 1398 AU/ml (95% CI 1169.6839 to 1627,2042) but this difference, although it exists, is not statistically significant $p = 0.123$. The cytomegalovirus-IgM level is lower in diabetic versus non-diabetic patients a statistically significant difference, $p = 0.001$. The *Helicobacter* IgG level is lower in diabetic patients with a mean value of 1.0763 U/ml with a 95% CI from 0.8141 to 1.3386 versus non-diabetics with an average of 1.3943 U/ml with a CI of 95% from 1.1963 to 1.5923, but this difference, although there, is not statistically significant, $p = 0.07$. The number of leukocytes diagnosed in diabetic patients is lower than that of people without diabetes ($p = 0.0001$). The level of C-reactive protein at diagnosis of diabetic patients is lower (an average value of 3.0207 mg/dl with a 95% CI of 0.9726 to 5.0688) than in people who do not suffer from diabetes (arithmetic mean of 5.8218 mg/dl with a 95% CI of 0.5894 to 11.0542), but this difference is not statistically significant. The serum level of fibrinogen at the diagnosis of diabetic patients is lower, with a mean value of 399.4 mg/dl with a 95% CI of 327.7993 to 471.0749, than that of people without diabetes, with an arithmetic mean of 653.8 mg/dl, with a 95% CI of 497.8700 to 809.8647, the difference being statistically significant, $p = 0.041$.

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Conclusions. *Inflammatory markers such as leukocyte levels at intake, C-reactive protein (CRP), and fibrinogen levels showed lower levels at admission in diabetic patients versus non-diabetic patients. There is no statistically significant difference between diabetic vs. non-diabetic patients regarding cytomegalovirus IgG levels, referring to cytomegalovirus IgM level is lower in diabetic versus non-diabetic patients with significant statistically difference. The Helicobacter IgG level is lower in diabetic patients versus non-diabetics patients.*

Keywords: inflammatory markers, infectious markers, ischemic stroke, diabetes

INTRODUCTION

According on the reports of the World Health Organization, stroke is the second main reason for death throughout the world, and the number of included subjects in stroke is drastically developing [1]. Stroke is one of the principal leading causes of death globally [2]. In 2005 stroke cause approximate 5.7 million death, 87% of deaths was in low and middle-income countries [3]. In 2016, according to “a systematic analysis for the global study on the burden of disease in 2016”, stroke remains the second leading cause of death in the world, with 5.5 million deaths [4]. Recently period, collecting evidence indicated that inflammation and atherosclerosis play important roles in stroke evolution [5,6].

Increased fibrinogen levels have been linked to early symptoms of atherosclerosis in asymptomatic patients [7]. Elevated fibrinogen levels might thus be a sign of atherosclerotic plaque development and progression, as well as contribute to it. In a numerous prospective studies, increased fibrinogen levels have also been found as a significant risk factor for future cardiovascular events [8-11].

Fibrinogen is an acute-phase protein that rises after a stroke [12], and high fibrinogen levels are linked to a higher risk of cardiovascular events in stroke survivors [12,13].

Increased levels of inflammatory cytokines [14] and C-reactive protein (CRP) are further indications of inflammation in ischemic stroke [15,16]. Increased CRP levels were linked to a worse short-term outcome in individuals with ischemic stroke in a recent research [17].

The inflammatory response has been linked to all phases of ischemic stroke and has been linked to the development of ischemia damage as well as the worsening of neurological function [18,19]. On the one hand, the number of leukocytes in the blood is linked to the severity of ischemia damage [20].

In acute ischemic stroke patients, early leukocytosis is linked to the amount of infarcted tissue [21]. Previous research, on the other hand, has found that leukocyte count is a major independent predictor of poor

clinical outcomes and discharge impairment [20]. It's also linked to a higher risk of all-cause death following an ischemic stroke [22]. Furthermore, a greater leukocyte count has been linked to a higher risk of recurrent ischemic stroke [23].

Human cytomegalovirus (human herpes virus 5) is a herpesviridae family double-stranded enveloped DNA virus. A increasing amount of in vivo and in vitro evidence supports the idea that human cytomegalovirus infection has a role in atherosclerosis progression [24,25].

Because human cytomegalovirus infection is generally diagnosed by serology [26], the question of whether human cytomegalovirus seropositivity is linked to the onset and progression of atherosclerotic lesions has become a important topic. Most previous epidemiological studies on human cytomegalovirus infection and atherosclerosis have relied on human cytomegalovirus-IgG, which is a marker of a long-standing immunological reaction resulting in an inflammatory response that, in many vascular pathobiological studies, would eventually explain advanced clinical atherosclerosis [26-28].

Helicobacter pylori (HP) is a Gram-negative microaerophilic bacteria with a helical structure. HP is a bacterial infection of the stomach mucosa that can last a lifetime and is most commonly acquired during childhood. *Helicobacter pylori* infection (HP-I) is a common infection in humans, and its incidence is linked to population age [29,30].

HP-I can induce persistent gastritis, peptic ulcers, and gastric cancer, among other things [29-31]. Furthermore, HP has been linked to coronary atherosclerosis both epidemiologically and pathogenetically, [32] although research on the link between chronic HP-I and ischemic stroke (IS) is debatable [33,34].

OBJECTIVE

The main aim of this study is to assess the plasma level of infectious and inflammatory factors in diabetic patients with acute ischemic stroke versus non diabetic patients with acute ischemic stroke.

MATERIALS AND METHODS

Study design

A prospective longitudinal observational study was conducted within the Clinical County Emergency Clinical Hospital of Oradea, Oradea, Romania, from 1st of January 2016 until 1st of January 2019. In the study, were enrolled 340 patients, both females and males with acute ischemic stroke.

The patients were divided into 2 groups: 101 diabetic patients (study group) and 239 non-diabetic patients (control group), were we analyzed as main criteria infectious factors (cytomegalovirus IgG plasma level, cytomegalovirus IgM plasma level, *Helicobacter pylori* IgG plasma level), inflammatory factors (leucocyte, C-reactive protein (CRP) and biochemical (plasma level of fibrinogen).

Determination of plasma levels of infectious factors were performed from the serum of patients at admission, by chemiluminescence method (CMIA), Architect ci4100 analyzer, inflammatory factors: C-reactive protein (CRP) by turbidimetry method, Architect c4000 analyzer, leukocytes whole blood by photometric impedance, analyzer Cell Dyn Ruby, biochemical factor: plasma level of fibrinogen was performed by the coagulometry method ACL TOP 550 CTS analyzer.

The inclusion criteria were as follows: age between 40 and 90 years, imaging-confirmed ischemic stroke diagnosis (CT-scan).

The exclusion criteria were represented by transient ischemic stroke, hemorrhagic stroke, hemorrhagic-transformed ischemic stroke, neoplastic patients, patients with autoimmune diseases, age over 90 years or under 40 years.

Ethical statement

The research was carried out with the agreement of the Ethics Commission of the Oradea County Emergency Clinical Hospital of Oradea (Oradea, Bihor County, Romania), no. 30372/06.12.2018 and according to the principles of the Declaration of Helsinki [35].

Statistical analysis

The database was collected in a Microsoft Excel document. The correlation analysis was carried out using the MedCalc 14.1 software where correlation tests included in the program were used. The correlation coefficient r , which can range from -1 and 1 was analyzed. A value r between -1 and 0 indicates an inversely proportionate correlation between the examined factors. A correlation coefficient value of 0 or close to it indicates that there is no correlation, whereas a value between 0 and 1 indicates a unilinear, directly proportional link. For each analysis, the Gaussian distribution of the data was studied, so as to use the Pearson coefficient, if it is observed, and the Spearman coefficient if

it is not observed. Results with a p value less than 0.05 were validated and considered statistically significant. These correlations were represented graphically by the graphical methods available in linear regression analyzes.

The limit of cut-off-point values was determined using ROC curves. The area under the curve, as well as the sensitivity and specificity of these values, are denoted as a consequence of this analysis. The p value, considered to be statistically significant, is a value below 0.05, which is obtained by comparing the area under the analyzed curves with an area under the curve of 0.5.

RESULTS

Cytomegalovirus-IgG levels are lower in diabetic versus non-diabetic patients with an arithmetic mean of 1170 AU/ml (95% CI 862.4169 to 1477.8326) versus 1398 AU/ml (95% CI 1169.6839 to 1627.2042) but this difference, although it exists, is not statistically significant $p = 0.123$. Both diabetic and non-diabetic patients often have cytomegalovirus-IgG levels between 0 and 3100 AU/ml. But non-diabetic patients appear to have high frequencies at higher levels (6,000-7,000 AU/ml and 9,000-10,000 AU/ml). A titer above this value has very few diabetic and non-diabetic patients with acute ischemic stroke (Figure 1).

FIGURE 1. Plasma level of cytomegalovirus IgG

Cytomegalovirus-IgM level is lower in diabetic versus non-diabetic patients with an arithmetic mean of 24.4145 Index (95% CI 20.2346 to 28.5943) versus 27.8820 Index (95% CI 23.3973 to 32.3667), a statistically significant difference, $p = 0.001$. Both diabetic and non-diabetic patients often have cytomegalovirus-IgM levels between 0 and 50 index, but diabetic patients appear to have a constantly higher cytomegalovirus-IgM level than non-diabetic patients (Figure 2).

Helicobacter-IgG level is lower in diabetic patients with a mean value of 1.0763 U/ml with a 95% CI from 0.8141 to 1.3386 versus non-diabetics with an average

FIGURE 2. Plasma level of cytomegalovirus-IgM

of 1.3943 U/ml with a CI of 95% from 1.1963 to 1.5923, but this difference, although there, is not statistically significant, $p = 0.07$. Both diabetic and non-diabetic patients most often have *Helicobacter*-IgG levels between 0 and 1 U/ml, but non-diabetic patients appear to have higher, constant frequencies compared to diabetics (Figure 3).

FIGURE 3. Plasma levels of *Helicobacter* IgG

Leukocytes number at diagnosis in the study group was generally between 6,000/ μ l and 12,000/ μ l, with an average of 8,672/ μ l and a standard deviation of 3,137.9. The highest leukocyte count is 18,000/ μ l, and the lowest is only 3,900/ μ l (Figure 4).

Leukocytes number diagnosed in diabetic patients with an arithmetic mean of 7,519.8614/ μ l with a 95% CI of 6,904.0321 to 8,135.6906 is lower than that of people without diabetes with a mean value of 9,159.1799/ μ l with a 95% CI of 8,774.0388 to 9,544.3210 ($p = 0.0001$) (Figure 5).

C-reactive protein level at diagnosis of diabetic patients is lower (an average value of 3.0207mg/dl with a 95% CI of 0.9726 to 5.0688) than in people who do not

FIGURE 4. Leukocyte count at admission

FIGURE 5. The value of leukocytes in diabetic patients versus in non-diabetic patients

suffer from diabetes (arithmetic mean of 5.8218 mg/dl with a 95% CI of 0.5894 to 11.0542), but this difference is not statistically significant (Figure 6).

FIGURE 6. Value of C-reactive protein at admission in diabetic patients versus non-diabetic patients

The serum level of fibrinogen at the diagnosis of diabetic patients is lower, with a mean value of 399.4 mg/dl with a 95% CI of 327.7993 to 471.0749, than that of people without diabetes, with an arithmetic mean of 653.8 mg/dl, with a 95% CI of 497.8700 to 809.8647, the difference being statistically significant, $p = 0.041$ (Figure 7).

FIGURE 7. Value of fibrinogen at admission in the diabetic patient versus the non-diabetic patient

Fibrinogen has a cut-off point that enjoys very good specificity and a sensitivity of 30.36%. Practically, 80% of patients with fibrinogen levels below 543 will not die. The area under the curve is not good here either, but the analysis has good power to predict survival with a 95% CI of 0.455 to 0.564 (Figure 8).

FIGURE 8. ROC curve – Fibrinogen – sensitivity, specificity

DISCUSSIONS

Human cytomegalovirus (human herpes virus 5) is a herpesviridae family double-stranded enveloped DNA

virus. A increasing amount of in vivo and in vitro evidence supports the idea that human cytomegalovirus infection has a role in atherosclerosis progression [24,25,36,37]. In the general population, however, there is evidence that human cytomegalovirus (HCMV) is associated to an elevated risk of cardiovascular disease (CVD). Chronic inflammation, dysregulated vascular function, and recurrent acute inflammatory reactions owing to periodic subclinical reactivation, according to mechanistic studies, might all be driving factors in accelerated atherogenesis [38].

The results of observational studies employing various cardiovascular disease (CVD) end points, on the other hand, are varied. Cytomegalovirus (CMV) infection was shown to be related with a slight elevated risk of CVD in a meta-analysis of ten prospective trials, but not with ischemic heart disease (IHD) or stroke in subtype analyses [39]. In study of Hamilton et al. they found no indication that HCMV infection was linked to an elevated risk of cardiovascular disease, ischemic heart disease, or stroke in the past. Age, sex, and other conventional cardiovascular risk variables were all revealed to be significant confounders in the HCMV-CVD association [40]. Past cytomegalovirus infection and stroke risk were studied in 14 studies, IgG cytomegalovirus seropositivity and/or high titre IgG antibodies were evaluated. When six case-control studies were combined, neither IgG seropositivity nor cohort studies were shown to be related with stroke [41-46]. In our research, cytomegalovirus-IgG levels are lower in diabetic versus non-diabetic patients but this difference, although it exists, is not statistically significant $p = 0.123$.

Cytomegalovirus (CMV) infection has been linked to an increased risk of cardiovascular disease, particularly in immunocompromised people [47]. A recent comprehensive analysis indicated that cytomegalovirus infection is linked to an increased risk of cardiovascular disease [39]. However, the implications of recent human cytomegalovirus infection as indicated by human cytomegalovirus immunoglobulin M (IgM) seropositivity in immunocompetent ischemic stroke patients are unknown. It's unclear if continuous human CMV infection is linked to an activated inflammatory state that leads to arterial plaque rupture. It's also unclear if recent human cytomegalovirus infection causes metabolic disorders during the acute phase of cerebral infarction [48].

Several research examining CMV infection (past or current) revealed that 19/22 studies had at least one category at high risk of bias, including confounding (ten studies had no age adjustment) and reverse causation (10 studies recorded CMV following stroke) [49].

In 11 case-control studies, the effects of recent CMV infection or reactivation were examined using a range of exposure criteria. IgM positive was linked to an in-

creased risk of stroke in a meta-analysis of two trials. IgM positive was linked to an increased risk of stroke in a meta-analysis of two trials [50]. There was extremely low-quality data showing no link between CMV infection and stroke in the past and an elevated risk of stroke after current CMV infection/reactivation [50-53]. Human cytomegalovirus-IgM has not been linked to acute ischemic cardiac or cerebral infarction in atherosclerotic patients [54]. In our research cytomegalovirus-IgM level is lower in diabetic versus non-diabetic patients with a statistically significant difference, $p = 0.001$.

Inflammation and infection by some microbial agents, particularly *Helicobacter pylori*, have been shown to modify several atherogenic vascular risk factors (e.g., homeostatic factors and lipids) in recent years [55,56]. Inflammation, immune-mediated vascular injury, direct bacterial invasion of atherosclerotic plaques, and hyperhomocysteinemia are among processes that contribute to *H. pylori* atherogenic risk [57].

Although several studies [58] have shown a link between antibody titers against *Helicobacter pylori* and coronary heart disease, the impact of *Helicobacter pylori* infection on key plasma biochemical risk markers for atherosclerosis and cerebrovascular disorders is still debated [33]. Although diabetes is associated with an increased risk of infection due to a compromised immune system, the seroprevalence risk of *Helicobacter pylori* infection in diabetic individuals is still debated [59].

In the study of Bures et al., *H. pylori* was shown to be less common in diabetics than in healthy people, according to certain research [60]. Xia et al. discovered no significant difference in *Helicobacter pylori* infection between diabetics and non-diabetics [61]. In our study, the *Helicobacter*-IgG level is lower in diabetic patients versus non-diabetics with but this difference is not statistically significant, $p = 0.07$.

Traditional risk factors for atherosclerosis, such as age, gender, race, ethnicity, hypertension, smoking, diabetes, hyperlipidemia, and hyperhomocysteinemia, have recently been discovered to be insufficient in explaining all clinical and epidemiologic features of atherosclerosis, as well as the incidence of its related vascular complications [62]. Several recent investigations suggested that an infection-induced inflammatory response might lead to widespread inflammation, a known risk factor for atherogenesis and ischemic vascular disease.

The fast development of damage in the afflicted zone(s) of brain tissue is a hallmark of stroke. Inflammation that occurs early in the course of acute cerebral ischemia accelerates the damage to the postischemic brain area, resulting in more necrotic tissue in the ischemic penumbra [63,64].

Rapid activation of resident cells, release of proinflammatory mediators, and infiltration of numerous

kinds of inflammatory cells, including leukocytes, define the acute and protracted inflammatory response [63,64]. Even 3 months after a stroke, peripheral inflammatory markers such as C-reactive protein and leukocytes are found to be at very high levels [65].

There is evidence that leukocytosis at the time of admission for a cerebrovascular incident is linked to the severity of the ischemia damage or the course of the ischemic damage. After multivariate analysis, a leukocyte count taken within 24 hours of start was strongly associated to initial stroke severity as measured by the Scandinavian Stroke Scale, but not to both clinical outcome and death at discharge in a sample of 763 patients [66].

In this investigation, Nardi et al. found that leukocytosis in the early stages of an ischemic brain event is a poor predictive indicator for severity, with poor evolution in the first 24 hours and greater impairment after discharge [20]. Other prior research have indicated that individuals with significant ischemic lesions and a high severity score exhibit considerable leukocytosis in the early stages of infarction, which supports the findings of this study [67]. Our data revealed the number of leukocytes diagnosed in diabetic patients is lower than that of patients without diabetes ($p = 0.0001$).

Furthermore, in study of Quan et al. they discovered that, while there was no significant difference in the relationship between leukocyte count and adverse clinical outcomes according to age, sex, history of hypertension, or smoking, the effect of leukocyte count on both short-term and long-term all-cause death was more pronounced among patients who had previously experienced a stroke or transient ischemic attack, and a similar result was found in patients who had previously experienced a stroke or transient ischemic attack, and a similar result was found [68].

Several animal studies have recently suggested that there are some distinct variations in post-ischemic inflammatory responses that may influence clinical outcomes. After an ischemic stroke, elderly, diabetic, and hypertensive animals showed increased inflammatory responses, which resulted in more severe brain damage and worse clinical consequences. The immunological mechanisms behind the aforementioned effects, however, are poorly understood. The cause of diabetes is chronic systemic low-grade inflammation, which manifests itself as reactive oxygen species production, proinflammatory cytokine release, and other inflammatory mediator activation [69].

Individuals with coexistence of hyperglycemia and raised biomarkers of inflammation had a higher risk of poor clinical outcomes among ischemic stroke patients, and those with elevated biomarkers of inflammation and hyperglycemia had a higher risk of short-term clinical outcomes [70].

C-reactive protein (CRP) is a well-known inflammatory marker. CRP levels in apparently healthy people may now be measured using newly developed high-sensitivity C-reactive protein (hs-CRP) tests. Even when variables known to be associated with increased CRP concentrations, such as infection and atherosclerosis, are eliminated [71]. CRP has been found to be linked to the risk of cerebrovascular events [72] and elevated in the circulation of patients following stroke.

Inflammation has a key role in diabetic vascular problems, according to Palem and Abraham [73]. Diabetic type 2 patients had a substantially high level of C-reactive protein (CRP) in previous investigations [74,75]. Our data revealed the level of protein C-reactive at diagnosis of diabetic patients is lower than in people who do not suffer from diabetes, but this difference is not statistically significant.

Following a stroke, inflammatory indicators such as CRP, adhesion molecules, and cytokines are among the most often expressed molecules. Inflammation is a key factor in all stages of atherogenesis. Acute-phase proteins have been linked to significant roles in ischemic stroke (IS) inflammatory processes, both acute and chronic. A number of studies have demonstrated the significance of acute-phase proteins as inflammatory indicators in IS [76].

By far the most important coagulation protein in the blood is fibrinogen, bulk, a precursor of fibrin and an essential component of blood viscosity and platelet aggregation are determined by this factor. Fibrinogen, a prothrombotic protein and acute phase reactant that is persistently increased in diabetic individuals, is one potential mechanism connecting diabetes to poor stroke outcomes [77].

This is the first study to show a connection between diabetic hyperfibrinogenemia and an increased risk of early neurologic degradation following an ischemic stroke. Increased fibrinogen levels are substantially and independently linked to the risks of coronary artery disease (CAD), stroke, and peripheral arterial disease, according to epidemiological research [78,79]. In our research, the serum level of fibrinogen at the diagnosis of diabetic patients is lower, than that of people without diabetes, the difference being statistically significant, $p = 0.041$.

Carotid artery stenosis is linked to elevated fibrinogen levels in stroke [80], and placebo data analysis in the Stroke Treatment with Ancrod Trial (STAT) and European Stroke Treatment with Ancrod Trial (ESTAT) found that plasma fibrinogen levels at stroke onset are independently linked to a poor functional outcome [81].

Several studies, notably in young and middle-aged men [80] showed that fibrinogen is a risk factor for is-

chemic stroke [82], whereas others found no such link [83].

The amount of fibrinogen in a patient's blood correlated with the extent of the infarction and their degree of awareness in acute stroke patients. At the same time, higher levels of fibrinogen are linked to more advanced atherosclerosis [84]. Beamer et al. found increased fibrinogen levels a year after a stroke [85]. We expand their findings by revealing that stroke survivors who had their stroke more than 2, 5, 7, or 10 years ago had similar inflammatory marker levels. These findings show that stroke survivors have low-grade inflammation that persists several years after the stroke [85].

The link between hyperfibrinogenemia and long-term mortality following stroke is far less well understood. In one research, ischemic stroke patients with hyperfibrinogenemia were more likely than those with normal plasma fibrinogen to die at 12 months, and the higher fibrinogen concentration was an independent predictor of mortality [86].

Our data revealed fibrinogen has a cut-off point that enjoys very good specificity and a sensitivity, the area under the curve is not good here either, but the analysis has good power to predict survival. Further studies with larger patient populations are needed to resolve these problems.

CONCLUSIONS

There is no statistically significant difference between diabetic vs. non-diabetic patients regarding cytomegalovirus Ig G levels, even if non diabetic patients appear to have high frequencies at higher levels. Referring to cytomegalovirus IgM level is lower in diabetic versus non-diabetic patients with significant statistical difference. The Helicobacter-IgG level is lower in diabetic patients versus non-diabetics patients.

Inflammatory markers such as leukocyte levels at intake, c-reactive protein (CRP), and fibrinogen levels showed lower levels at admission in diabetic patients versus non-diabetic patients. Fibrinogen has a cut-off point with strong specificity and sensitivity. Although the area under the curve is not good but the analysis shows strong predictive power for survival.

This vast subject requires more extensive studies on a larger number of patients, due to present pro and other cons studies, with the focus of this issue on the pathology of the diabetic patient.

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