A Rigorous Study on Relation of Stroke Risk Factor With Specific Ischemic Brain Stroke in Indian Patients: A Case Control Study

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Research

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Abstract

Background

Recent evidences suggested that deficiency of Vit-D is associated with risk of ischemic stroke and its severity. The mechanism behind its relationship have been linked to total oxidant level and some other modifiable risk factor like hypertension, diabetes, smoking and alcohol consumption and tobacco chewing. However, there are disappearances in evidences. The aim of this study is to find out the role of Vit-D and Total oxidants in ischemic stroke and in disease progression.

Methods

In this case control study total 205 individuals of case and control enrolled in this study the demographic data like age, gender, ethnicity, dietary habit, addiction (smoking, alcohol, tobacco chewing) previous history of disease (hypertension, diabetes, any cardiovascular disease) were collected from family members and other medical record.

Results

The mean age of case and control group of 63.91 ± 12.66 and 62.52 ± 7.04 respectively. There was significant difference between insufficient Vit-D level in case and control group ($p<0.05$) however, there were significant difference of MDA in the groups classified on the basis of Vit-D level in case and control ($p<0.05$) moreover, there were inverse association observed between Vit-D and MDA level in both case and control. Vit-D and MDA shows significant association ischemic stroke however, MDA have association with stroke severity.

Conclusion

The result shows higher prevalence of Vit-D deficiency in ischemic stroke patients which induce MDA level parallelly. At low level of Vit-D and high level of MDA associated with the severity of stroke. Moreover, Vit-D and MDA shows significant association with stroke occurrence. Furthermore, MDA significantly associated with stroke severity however Vit-D does not show any association with stroke severity

Background

Stroke is a rapid clinical setting and neurological deficit which associate to a focal injury of brain including cerebral infraction (ischemic stroke)and intracerebral or subarachnoid hemorrhage (hemorrhagic stroke) by vascular cause and it is a major cause of death and disabilities through worldwide [1].

According to world stroke organization report 5.5 million of death and 116.4 million disability-adjusted life years (DALY) occurred due to stroke till 2016. The worldwide age-standardized mortality rate of

ischemic stroke is decrease from 1990 to 2016 by rate of 36.2 % and age-standardized DALYs rate is also reduced over the same period by 34.2 % in high income countries [2]. In developing countries like India, stroke is leading cause of death and disabilities. In rural area the estimated adjusted prevalence rate of stroke range0.08-0.26 % in rural and 0.33-0.42%inurban area [3].

The pathophysiology of ischemic stroke is very complex, approximately 80-85 % of ischemic stroke characterized by cerebral blood flow disruption and deficiency of oxygen. The deficiency of oxygen creates oxygen imbalance in brain cells which might be generate oxidative stress [4]. Several authors evidently proof that the high level of oxidative stress provoke the risk of ischemic stroke [5]. Aygul et alsuggested, nitric oxide (NO) and MDA (marker of Total oxidant) level has been increased in acute ischemic stroke patients[6]. Içme et al reported,Total oxidants plays major role in ischemic and haemorrhagic stroke however, it does not show any correlation with stroke severity [7].

Vit-D is essential component for humans, it plays critical role in bone metabolism [8]. There has been increasing interest to identify the relationship in Vit-D in extra-skeletal disease [9], because of the receptor of Vit-D are present in various cells and organ including brain and other immune cells [10-12]. Moreover, Vit-D is also involved in regulation of neurotrophic factor and maintenance of blood brain barrier integrity [13]. Some author evidently proof that sufficient Vit-D level reduces the risk of ischemic stroke by decreasing the fatal effect of modifiable risk factor (hypertension, diabetes, smoking and alcohol consumption) [14-16].

The level of Vit-D and oxidative stress in ischemic stroke patients have been accessed by many authors,Park et alfound that low serum Vit-D level could be a good predictor for ischemic stroke [17]. The incidence of stroke has been increase in among old age woman which having serum Vit-D level < 50nmol/L [18]. In Japanese-American men low dietary intake of Vit-D was independent risk factor for all type stroke [19]. Gupta et al observed,there is no association of Vit-D deficiency with ischemic stroke [20].However, due to various cofounding factors, there are disappearances in association of Vit-D level and Total oxidant level in ischemic stroke patients. So, at present it is necessary to identify the role of Vit-D and Total oxidantsin ischemic stroke and it severity.The aim of this study is to find out the role of Vit-D and Total oxidants in ischemic stroke and in disease progression.

Subjects And Methods

In this hospital-based case control study, Total 205 individuals were enrolled in which 101 patients were recruited as a case who diagnosed with first ever ischemic stroke on the basis of their CT/MRI report and admitted within 24 hours after stroke in KPS institute of Neurology, GSVM Medical Collage, Kanpur, rest 104 age and sex match healthy ambulatory individuals was also enrolled and assign as control.

This study was approved by Ethical Committee of GSVM Medical college Kanpur.

Data and Sample Collection

After enrolment of cases and controls, clinical and demographic data including age, gender, ethnicity, dietary habit, addiction (smoking, alcohol, tobacco chewing) and previous history of disease (hypertension, diabetes, cardiovascular disease) were collected from family members and other medical record.

Under aseptic condition 5 ml venous blood had been drawn from the antecubital vein, 3 ml blood collected in (Ethylenediaminetetraacetic acid)EDTA vial and rest 2 ml blood collected in plain vial, after centrifugation plasma and serum will be stored at −80°C until analysis of Vit-D and Total oxidants.

Serum 25 Hydroxy Vit-D was measured by chemiluminescence (CLIA) and classified in Deficient (<20ng/ml), insufficient (20-29 ng/ml) and sufficient level (>29 ng/ml)[21].Malondialdehyde (MDA) is major marker of lipid peroxidation as well as Total oxidants in human serum and it was measured by thio-barbituric acid reactive substances (TBARS) method.

National Institutes of Health Stroke Scale (NIHSS)

The general and neurological examination was done for assessment of neurological function, within 24 hr of admission of patients using NIHSS score by neurologist. NIHSS score is a neurological examination which directly reveals stroke severity. It has 11 parameters which used in evaluation the effect of ischemic stroke on level of consciousness, eye movement, visual field, movement of arm and legs, ataxia, dysarthria, and sensory loss. The NIHSS consist of five section, 0 score (No Stroke), 1-4 (Minor), 5-15 (Moderate), 16-20 (Moderate to Severe stroke) and < 21 (Severe stroke) [22].

Statistical analysis:

Generated data were analysed by statistical program for social science (SPSS) version 22, IBM Corp., Chicago, USA, 2013. Quantitative data and qualitative data were express in Mean ± SD, frequency and percentage respectively. Independent t-test was used to compare between two independents for quantitative data. For comparing more than two variable ANOVA was used. For establishment correlation between variables, Pearson’s correlation was used. All statistical test was performed at significance level of <0.05.

Results

The mean age of case and control group of 63.91 ± 12.66 and 62.52 ± 7.04 p value = 0.33 respectively, age is not significance. There were male 61.38 % and 65.04% and 38.61% and 34.95% female in both case and control group respectively. There was highly significant difference between smokers, tobacco chewer and alcohol consumers in case and control group. Moreover, in this study 67 (66.33%) patients were hypertensive in case group and 23 (22.23%) individuals in control group and the difference between case and control group is highly significant is decribed in Table 1.

In this study Table 2 total deficient 60 (59.40%), insufficient 26 (25.74%) and rest 15 (14.84%) have sufficient level of Vit-D in case group, in the same way 32 (30.76%) deficient, 37 (35.77%) insufficient and

35 (33.65%) individuals having sufficient level of Vit-D in control group. There was significant difference between insufficient Vit-D level in case and control group however, there were significant difference of MDA in the groups classified on the basis of Vit-D level in case and control. Interestingly,with the increasedVit-D level, MDA level decreased in both case as well as control group.So, there was inverse association detected between vitamin and MDA level.

In univariate analysis Vit-D and MDA were significanly associated with the risk of ischemic stroke. Moreover in multivariate analysis MDA were associated with ischemic stroke occurance is decribed in Table 3.

To find the factor which is responsible for strokeseverety, the logistic regression analysis has been performed in this study. Serum Vit-D do not have any association in both univariate and multivariate level but serum MDA significantly associated with risk of stroke severety in both univariate and multivariate analysis is decribed in Table 4.

Shows figure.1 the relationship between Vit-D and MDA level with stroke severity. Stroke severity was measured by NIH stroke scale.We observe there are 7 (6.93%) minor, 28 (27.27%) moderate, 10 (9.90%) moderate to severe and rest 56 (55.44%) severe stroke patients. Interestingly, with the decreased Vit-D level, the level of MDA increased in stroke severity in the groups categorized on the basis of NIH score scale.

Discussion

In present study we investigate the association betweenserum Vit-D level and Total oxidant with ischemic stroke patients compared with control group. As per our result 59 % of stroke patients and 30% of healthy control individuals have severe Vit-D deficiency indicates that majority of population had low level of Vit-D (Table 2).

We also found that there was no significant difference in Vit-Dlevel in deficient and sufficient groups in case and control group (Table-2). These results are in accordance with the previously publised studies. Chaudhuri et al reviled that Vit-D deficiency is an independent risk factor for large artery atherosclerosis of ischemic stroke and cardioembolic stroke patients [23]. Afshari et al found that severe Vit-D deficiency and lower fruit intake induces the risk of ischemic stroke [24]. Narasimhan et al compared stroke outcome on Vit-D supplementation in ischemic stroke patients and conclude that severe deficiency of Vit-D induce risk of stroke and replenishment of Vit-D for three months improve the stroke outcome [25].Baser et al reported increase level of Total oxidant and Vit-D deficiency favours the risk of ischemic stroke [26].Tsai et al reported that large vessel diseased ischemic stroke having high level of thio-barbituric reactive substance (marker of oxidative stress) and low level of free thiol group (anti-oxidant level marker) in ischemic stroke patients[27].An animal-based study suggested that Vit-D supplementation can reduced lipid peroxidation and oxidative stress induced tissue damage [28]. Sepidarkish et al observe that Vit-D supplementation can reduce the toxic effect of oxidative stress in ischemic stroke patients [29].

In our study we also access the risk factor for occurrence of ischemic stroke through univariate and multivariate logistic regression analysis. We found that Vit-D and MDA was significantly associated the risk of stroke in univariate analysis. Moreover, in multivariate analysis MDA shows significant association with risk of ischemic stroke occurrence. Huang et al reported that deficient level of Vit-D is major and potential risk factor for stroke related disease like stroke associated pneumonia [30]. A meta-analysis done by Zhou et al reported that low level of Vit-D significantly increase risk of ischemic stroke [31].

However, other risk factor like age, gender, smoking, tobacco chewing, alcohol comsumption, hypertension were significantly provoke the risk of stroke in univariate analysis and in multivariate analysis exept tobacco chewing. Mallmann et al addresses the different risk factor associated with ischemic stroke and conclude that atrial fibrillation, left ventricular hypertrophy, hypertension, physical inactivity, low levels of HDL-cholesterol, smoking, carotid bruit, diabetes, alcohol abuse significantly induce the risk of stroke with different propensities [32].

Interestingly, in our study we also address the risk factor which affect the strokeseverity by logistic regression analysis (Table no. 4) and found that serum MDA level was significantly associated with the risk of stroke severity in both univariate and multivariate analysis. Moreover, serum Vit-D and other factors like smoking, alcohol consumption, tobacco chewing, diabetes and hypertension does not have any association with stroke severity in both univariate and multivariate analysis.Wang et al stablish significant negative correlation between stroke severity and serum Vit-D level [33]. Ferretti et al and Nanetti et al investigate the relation between level of oxidative stress and ischemic stroke event and reviled that impairment in oxidative stress and antioxidants system might be play significant role in stroke event [34-35]. Lorenzano et al evaluate the role of oxidative stress, inflammation and tissue damage and correlate them with infarct growth in ischemic stroke patients and found that elevated level of oxidative stress independently predict the growth of infraction in ischemic stroke patients [36].

Conclusion

The result shows higher prevalence of Vit-D deficiency in ischemic stroke patients which induce MDA level parallelly. At low level of Vit-D and high level of MDA associated with the severity of stroke. Moreover, Vit-D and MDA shows significant association with stroke occurrence. Furthermore, MDA significantly associated with stroke severity however Vit-D does not show any association with stroke severity.

LIMITATIONS OF THE STUDY

The main limitation of present study is sample size. Larger clinical studies in this area are needed to establish the relationships between Vit-D and Total oxidant level in ischemic stroke patients.

Declarations

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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Authors' contributions

In this article, Dharmsheel Shrivastav, Manish Kumar Verma, Pratibha Tripathi, Nivedita Saxena, performed the experiments, and wrote the manuscript. Desh Deepak Singh designed manuscript. Alok Verma & J S Kushwaha provide clinical information material. Jay Prakash support in statistical analysis. Anand Narayan Singh study design and added valuable suggestions to improve the manuscript, validation and supervision.

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Tables

Table No. 1: Base line characteristics of case and control group.

S.NO.	Parameter		Case (n=101)	Control (104)	P-value*
1.	Age (Mean± SD)		63.91 ± 12.66	62.52 ± 7.04	0.33
2.	Gender	Male	62 (61.38%)	67 (65.04%)	0.58
		Female	39 (38.61%)	37 (34.95%)	0.55
3.	Addiction	Smokers	37 (36.63%)	27 (25.96%)	<0.01*
		Tobacco chewer	79 (78.21%)	51 (49.51%)	<0.01*
		Alcoholic	36 (37.62%)	24 (23.30%)	0.05
4.	Diabetic		31 (30.69%)	25 (24.27%)	0.34
5.	Hypertensive		67 (66.33%)	23 (22.33 %)	<0.001*

* Significant association p Value <0.05

Table: 2 ONE WAY ANOVA for Serum Vit-D and MDA level between case and control

Parameter		Case (101)		Control (104)		P value*
		Count	Mean ±SD	Count	Mean ±SD	
Vit-D	Vit-D **Deficient**	60 (59.04%)	11.6±4.7	37 (35.57%)	13.2±5.4	0.14
Vit-D	Vit-D **insufficient**	21 (20.79%)	24.1±3.21	38 (36.53%)	27.3±6.8	0.01*
Vit-D	Vit-D **sufficient**	20 (19.80%)	33.6±4.4	29 (27.88%)	35.9±4.15	0.07
MDA	Vit-D **Deficient**	60 (59.04%)	12.7±6.7	37 (35.57%)	7.4±5.04	0.00*
MDA	Vit-D **insufficient**	21 (20.79%)	10.8±5.4	38 (36.53%)	6.2±4.0	0.00*
MDA	Vit-D **sufficient**	20 (19.80%)	10.2±6.7	29 (27.88%)	3.7±2.7	0.00*

Significant association p Value <0.05

Table :3 Logistic regression analysis for Stroke Occurrence

Parameter	Univariate logistic regression			Multivariate logistic regression		
	ODD Ratio	95% CI	P Value*	ODD	95% CI	P Value
Age	0.91	0.89-0.94	<0.00*	0.90	0.86-0.94	<0.00*
Gender	0.28	0.14-0.56	<0.00*	0.19	0.05-0.76	0.01*
Smokers	10.47	5.46-20.07	<0.00*	5.16	1.52-17.46	<0.00*
Alcoholic	14.82	7.47-29.39	<0.00*	9.61	2.51-36.74	<0.05*
Tobacco Chewer	4.91	2.54-9.50	<0.00*	3.12	0.72-13.40	0.12
Diabetes	0.78	0.42-1.43	0.425	0.28	0.07-1.10	0.06
Hypertension	14.38	7.31-28.28	<0.00*	21.24	5.68-79.44	<0.05*
Vit-D	1.05	1.02-1.08	<0.05*	1.00	0.95-1.06	0.81
MDA	0.81	0.76-0.87	<0.05*	0.76	0.66-0.87	<0.05*

Significant association p Value <0.05

Table-4 Logistic regression analysis for Stroke severity (NIHSS score scale)

Parameter	Univariate logistic regression			Multivariate logistic regression		
	ODD Ratio	95% CI	P Value*	ODD	95% CI	P Value*
Age	1.00	0.97-1.04	0.66	0.99	0.96-1.03	0.82
Gender	1.17	0.50-2.73	0.70	1.18	0.46-3.03	0.72
Smokers	1.41	0.51-3.86	0.50	1.63	0.48-5.48	5.48
Alcoholic	0.55	0.16-1.85	0.33	0.28	0.70-1.16	0.08
Tobacco Chewer	1.22	0.40-3.70	0.72	1.62	.044-5.89	0.46
Diabetes	0.94	0.36-2.41	0.90	0.87	0.31-2.45	0.79
Hypertension	1.18	0.42-3.36	0.74	1.56	0.49-4.98	0.44
Vit-D	0.96	0.92-1.01	0.15	0.97	0.93-1.02	0.25
MDA	1.00	0.97-1.04	0.00*	1.10	1.02-1.18	0.00*

Significant association p Value <0.05

Figures

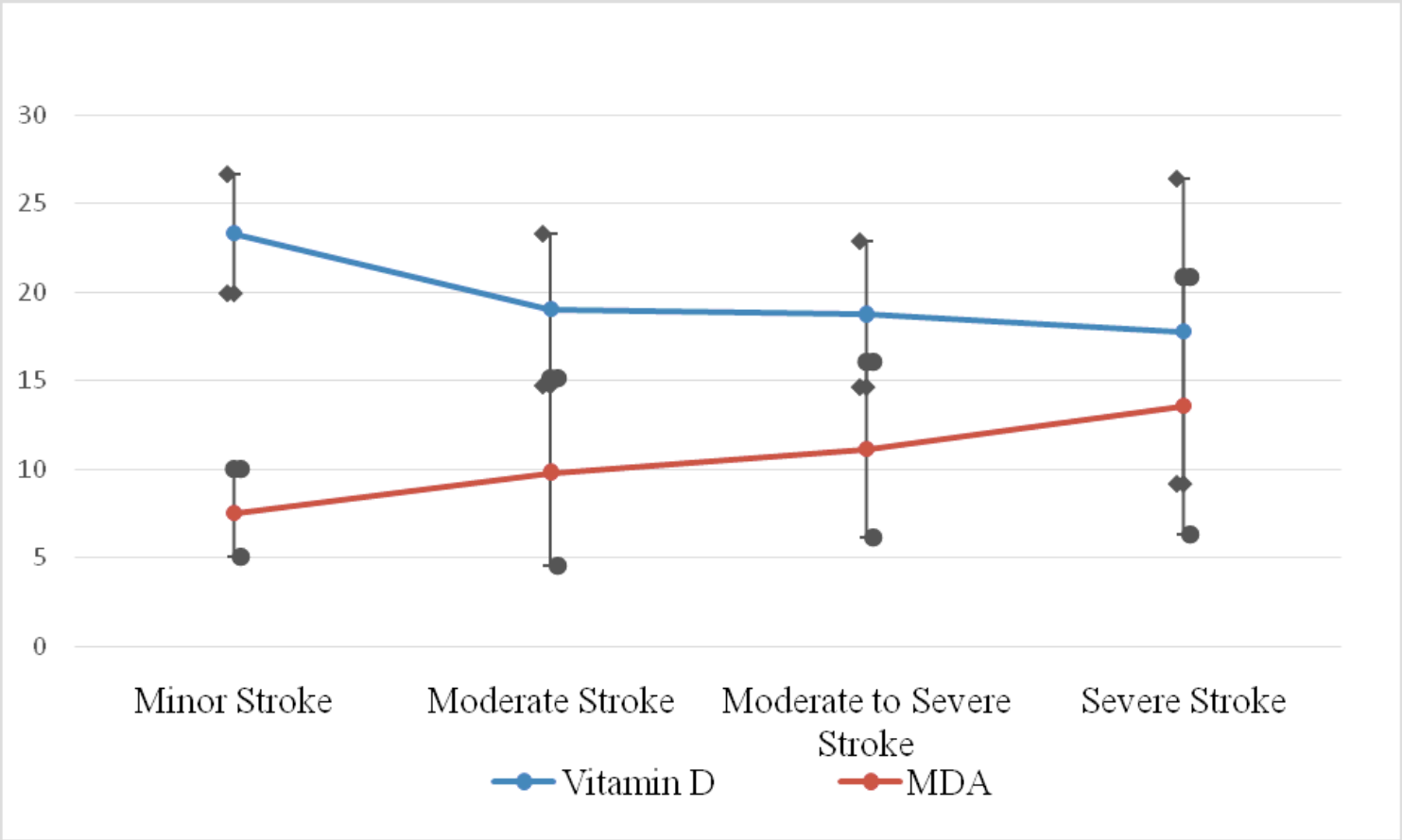

Figure 1

Effect of Vit-D and MDA in association with Stroke severity