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Vitamin D and Stroke: A Comparative Study to Risk Factors and Stroke Type

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Abstract
Background: *Stroke remains one of the most devastating neurological diseases, often causing death, or gross physical impairment. Vitamin D deficiency has been reported to contribute to the risk of cardiovascular disease especially stroke. Thus, our study was designed to examine the relationship between serum 25 (OH) D levels and outcomes after stroke either ischemic or hemorrhagic.*
Methods: *Fifty patients with first–ever acute stroke (25 patients with intracerebal hemorrhage and 25 patients with ischemic infarction). All participants were subjected to full clinical and neurological examination. Brain imaging was performed. Blood samples were drawn for assessment of serum 25-hydroxyvitamin D and parathormone.*
Results: *The Majority (84% and 76% respectively) of our studied patients with acute stroke had insufficient levels of vitamin D (< 50 nmol/L) with more reduction in patients with acute ischemic infarction than hemorrhagic cases (28.72 ± 22.45 versus 38.49 ± 24.68 in group I and group II respectively), but with no statistical significance differences between both groups. There was an association between vitamin D levels, severity of stroke and functional outcome in patients with intracerebral hemorrhage but not in patients with ischemic stroke. In dead patients with intracerebal hemorrhage, an insufficient median vitamin D level was noticeable.*
Conclusion: *Vitamin D insufficiency may bear an association with acute stroke and its prevalent risk factors with a highest association between low 25(OH)D levels and risk of incident hemorrhagic strokeamong people in Upper Egypt.*
Keywords: *Acute stroke, Scandinavian Stroke Scale (SSS), Modified Rankin Scale (mRS), Serum 25-hydroxyvitamin D, Parathormone (PTH).*

Introduction
The classic role of vitamin D as a regulator of calcium level that affects bone, neuron, and muscle functions has been recently extended by reports that show that vitamin D deficiency might be involved in the development several diseases, including hypertension, diabetes mellitus and heart failure[1]. Vitamin D has pleiotropic effects that may favorably influence cardiovascular health through multiple mechanisms including down regulation of renin-angiotensin system, enhance-ment in insulin secretion and insulin sensitivity, protection against angiogenesis and modulation of inflammatory processes[2]. Accumulating evidence suggests that vitamin D deficiency is associated with increased risk of stroke. Contributing

mechanisms have been linked to the association of vitamin D deficiency with the presence of hypertension, diabetes mellitus, atherosclerosis, thrombosis and inflammation, however, the evidence is conflicting [1,3-6]. Hence, it was interesting to explore the association between the vitamin D deficiency with increased risk of stroke by the possible explanations like the vascular effects of vitamin D deficiency, that includes modulation of smooth muscle cell proliferation[7], a potential interaction between vitamin D deficiency and hypertension[8], inflammation[9] , thrombosis[10]. Also, the scientific facts that vitamin D deficiency triggers secondary hyperparathyroidism which promotes myocyte hypertrophy, vascular remodeling and has proinflammatory effects[8].

Furthermore, Vitamin D may play a role in neuroprotection, perhaps through detoxification pathways, inhibition of inducible nitric oxide synthase, antioxidation/anti-inflammatory mechanisms, neuronal calcium regulation or enhanced nerve conduction[11]. Pilz etal[1] and Witham et.al.[12] stated that 25(OH) D can crosses the blood brain barrier, therefore, it has been suggested that vitamin D receptors (VDR) activation may exert some neuroprotective effects that may be beneficial in stroke patients. Thus, high dose oral vitamin D supplementation produced short-term improvement in endothelial function in stroke patients. Data from a population-based study showed that elderly persons with a low intake of vitamin D and low serum concentrations of 1, 25 (OH)2D were at increased risk for future strokes even after adjustment for age, sex, smoking and functional capacity. In the present study, the primary outcome was the relation of Vit D level and the outcome after stroke either ischemic or hemorrhagic in the general population from Upper Egypt. The secondary outcomes were the association of Vit Dand parathormone level with stroke severity, stroke risk factors and stroke types.

Materials and Methods
Participants

This observational prospective study was conducted on fifty patients with first –ever acute onset stroke within seven days (25 patients with intracerebral hemorrhage and 25 patients with ischemic infarction; their ages ranged 20 – 87 years old. Patients were eligible for inclusion if they were admitted to our units with acute stroke with symptom onset within 24-48 hours which was confirmed by brain imaging (either CT scan and /or MRI brain). We excluded patients with brain neoplasm, history of previous stroke, hepatic and renal impairment, endocrinal diseases, vitamin D or Ca supplementation, previous fractures, steroid therapy, malignancy and bone diseases. The study has been approved by the ethical committee of Faculties of Medicine, Assiut University and Aswan University and a written informed consent was obtained from each participant relatives. All participants were subjected to thorough history taking and full clinical examination. Complete neurological examination where the following clinical and demographical data were taken; age sex, presence of stroke risk factors (as smoking, history of hypertension, history of transient ischemic attacks, diabetes mellitus, history of myocardial infarction or any cardiac arrhythmia and hyperlipidemia). Stroke severity was assessed by Scandinavian Stroke Scale (SSS) and functional outcome was determined according to Modified Rankin Scale (mRS) that was measured at the time of admission. Brain imaging (either CT scan and /or MRI) was performed at admission. Electrocardiography (ECG), Echocardiography (ECHO), Carotid and vertibrobasilar duplex were done for all patients. 10 ml of venous blood was collected from all studied patients; 2 ml in EDTA tube for complete blood picture (has been done on sysmex XP-300 cell counter) and 8 ml divided on two plan tubes (for serum sample collection) for routine and special parameter estimation ; Serum collected samples had been left for clotting. All serum samples were centrifuged at

3000 rpm for 20 min after clotting. _Serum was separated and divided into 3 divisions; one for routine parameter estimation (random blood glucose, renal and liver function tests, thyroid function tests, lipid profile, calcium and phosphorus), all parameters were done on fully automated chemistry analyzer BT-3500 (Italy). The other 2 divisions were freezed at $-20\,℃$ for later use for estimation of 1, 25-dihydroxy Vitamin D and Parathormone (PTH) using a Sandwich ELISA technique (Calbiotech Inc. 10461 Austin Dr, Spring Valley, CA). All blood samples were collected on the first day of admission and hemolyzed samples were excluded.

Statistical analysis

Data was computerized and analyzed using the (SPSS 20.0 software, Chicago, IL, USA) computer program. The normality of the data was assessed by Kolmogorov-Smirnov test. appropriate. Chi-square test and Fisher exact test was used to compare between categorical variables. Continuous variables were compared by t-test (normally distributed data) and Mann-Whitney (not normal distributed data). A two-tailed $p < 0.05$ was considered statistically significant. Data was presented as mean ± SD, median (range) or numbers and percentages when appropriate. Chi-square test and Fisher exact test was used to compare between categorical variables. Continuous variables were compared by t-test (normally distributed data) and Mann-Whitney (not normal distributed data). A two-tailed $p < 0.05$ was considered statistically significant. The best cut-off was defined on the basis of analysis of the ROC curves by identifying the value of the biomarker that gave the best combination of sensitivity and specificity, that is, the value that maximized the sum of the sensitivity and specificity. The ROC curve analysis was performed using the MedCalc software version 7.50 (Mariakerke, Belgium).

Results

The baseline demographic and clinical characteristics of studied acute stroke patients are shown in Table (1). No statistically significant differences regarding the ages of patients and gender were found between both studied groups. However, there was less duration of onset of acute stroke in patients of group II (9.5±8.67 hours) when compared to that of patients of group I (18.9±10.87 hours) with statistical significant difference ($p < 0.001$). An unfavorable functional outcome (100 %) was found in patients of group II with mRS score of 4.6±0.58 when compared to that in group I (96%) with MRS score of 4.4±0.76. However, there was no significant difference between both studied groups. Scandinavian Stroke Scale (SSS)was 25.3±14.9 versus 25.5±15.8 in group I and group II respectively. Modified Rankin scale was 4.4±0.76 in group I while it was 4.6±0.58 in group II. Both scales showed in significant differences between both groups ($p > 0.5$). The number of deaths was fairly equal in both groups with no statistically significant difference. As regarding risk factors; the highest proportion of smoking (40%), DM (36%), Dyslipedemia (64%) and non valvular AF (16%) were found among group I patients. While hypertension (76%) and ischemic heart diseases (24%) were found higher among group II patients. The abnormal findings in the carotid (68% in group I versus 24% in group II, with $p < 0.001$) and vertibrobasilar duplex (24% in group I versus 4% in group II, with $p = 0.04$) were recorded. The most common abnormalities were carotid and vertibrobasilar atherosclerosis. Carotid stenosis was found 12% in group I and 8% in group II.

Table 1: Basic clinical characteristics of studied patient groups.

Parameters	Patients with Ischemic Stroke (Group I) (No =25) Mean ±SD	Patients with intra cerebral Hemorrhage stroke (Group II) (No =25) Mean ±SD	P-Value
Age , years (mean ± SD)	60.56±9.89	61.32±12.86	0.816
Sex (male/female) (%)	11/14 (44% /56%)	14/11 (56% / 44%)	0.572
Duration of stroke (Hours) (mean ± SD)	18.9±10.87	9.5±8.67	0.002
Affected side (%) Right Left	9 (28%) 16 (72%)	15 (60%) 10 (40%)	0.61
Modified Rankin scale (mRS) (mean ± SD)	4.4±0.76	4.6±0.58	0.214
Scandinavian stroke scale (SSS) (mean ± SD)	25.3±14.9	25.5±15.8	0.964
Unfavorable outcome (%)	24 (96%)	25 (100 %)	0.5
Number of deaths(%)	6 (24%)	6 (24%)	1.0
Blood pressure (mean ± SD) SBP (mm Hg) DBP (mm Hg)	144.8±17.34 84.8±7.7	159.2±14.8 93.6±10.5	0.003
Risk factors: (%) Hypertension Dyslipidemia Diabetes Mellitus Smoking Ischemic heart disease Atrial fibrillation	16 (64%) 19 (76%) 9 (36%) 10 (40%) 3 (12%) 4 (16%)	16 (64%) 16 (64%) 7 (28%) 8 (32%) 6 (24%) 3 (12%)	1.0 0.27 0.76 0.79 0.46 1.0
Carotid duplex Normal (%) Abnormal (%)	8 (32%) 17 (68 %)	19 (76%) 6 (24 %)	0.001
Vertibrobasilar duplex Normal (%) Abnormal (%)	19 (76%) 6 (24%)	24 (96%) 1 (4%)	0.04

*P-value < 0.05

The higher levels of lipid profile were found in patients of group I when compared to that in patient group II but with no statistical significance differences , While the highest levels of random serum glucose and serum creatinine were found in studied patient group II but with no statistical significance differences, Notably, the mean levels of total protein, serum album , thyroid function tests, serum total Ca and phosphorus were nearly similar in both studied groups with no statistical significance differences as shown in Table (2). The Majority of our studied stroke patients had insufficient levels of vitamin D (< 50 nmol/L) with more reduction in patients with acute ischemic infarction but with no statistical significance differences between both groups. Notably, the highest levels of parathormone in hemorrhagic patients group were found with no statistical significance differences, Table (2) and Figure (1)

Table 2: Basic laboratory characteristics of studied patient groups.

Parameters	Patients with ischemic Infarction (Group I) (No =25) Mean ±SD	Patients with intra cerebral hemorrhage (Group II) (No =25) Mean ±SD	P-Value
Vitamin D level (nmol)	28.72 ± 22.45	38.49 ± 24.68	0.15
Vitamin D status			
Sufficient(> 50 nmol/L)	4 (16%)	6 (24%)	0.36
Insufficient (<50nmol/L)	21 (84%)	19 (76%)	
Parathormone hormone (i PTH)(ng/l)	57.34 ± 63.76	65.90 ± 62.58	0.63
Serum total Calcium level (mg/dl)	9.30 ± 1.34	9.17 ± 1.63	0.74
Serum phosphorus level (mg/dl)	4.71 ± 1.09	4.70 ± 0.85	0.97
Total cholesterol (TC) (mmol/L)	214.89 ± 50.68	194.54 ± 35.89	0.11
LDL-C (mmol/L)	141.83 ± 59.38	123.84 ± 22.01	0.16
HDL-C (mmol/L)	48.58 ± 10.68	48.24 ± 5.25	0.89
Triglycerides (TG) (mmol/L)	132.5 ± 51.71	126.12 ± 40.89	0.63
Random blood glucose (RBG) (mg/dl)	144.32 ± 70.44	162.45 ± 96.137	0.45
Free T 3	3.33 ± 0.96	3.15 ± 0.77	0.47
Free T4	18.6 ± 4.49	17.10 ± 3.56	0.21
TSH	0.76 ± 0.61	0.81 ± 0.58	0.75
Erythrocyte sedimentation rate (ESR)	54.28 ± 22.10	67.92 ± 20.89	0.03[*]
Creatinine(mg/dl)	1.07 ± 0.51	1.81 ± 3.39	0.28
Total protein (gm/l)	7.82 ± 0.93	8.34 ± 0.92	0.05[*]
Serum albumin (gm/l)	4.62 ± 0.78	4.51 ± 0.84	0.63

*P-value < 0.05

Table 3 a and b shows the basic laboratory characteristics between the survivors and deaths in both studied patients groups: The mean levels of serum total Ca and phosphorus were nearly similar in both survivors and died with no statistical significant difference between both groups. Meanwhile, the statistically significant lower levels of vitamin D were found in deaths of both studied groups. Notably, patients of group I showed lower levels of iPTH in survivors when compared to those levels in deaths with statistical significant difference. Nevertheless, in patients group II, the results demonstrated a higher level of iPTH in survivors when compared to that level in deaths but with no statistical significant difference.

Table 3 (a) : Serum level of calcium and phosphorus in survivors and deaths in studied patients groups.

Parameters	Patients with ischemic Infarction (Group I) (No =25) mean ± SD			Patients with intracerebral hemorrhage (Group II) (No =25) mean ± SD		
	Survivors No = 19	Deaths No.=6	P-Value	Survivors No = 19	Deaths No.=6	P-Value
Serum total Calcium (mg/dl)	8.65 ±21.33	8.97 ±27.94	0.98	9.19 ±1.69	9.10 ±1.55	0.91
Serum Phosphorus (mg/dl)	4.49 ± 1.12	4.73 ± 1.90	0.39	4.57 ± 0.91	5.10 ±0.51	0.09

*P-value < 0.05

Table 3(b) : Vitamin D status and serum intact parathormone levels (i PTH) in survivors and deaths in studied patients groups.

Parameters	Patients with ischemic Infarction (Group I) (No =25) Median			Patients with intracerebral hemorrhage (Group II) (No =25) Median		
	Survivors	Deaths	P-Value	Survivors	Deaths	P-Value
i PTH (ng /ml)	26.70 (52.70)	55.30 (70.80)	0.001*	55.60 (63.50)	35.70 (81.25)	0.594
Vitamin D (nmol/L)	22.10 (23.80)	14.30 (54.35)	0.93	44.20(53.50)	18.85 (16.55)	0.003*

*P-value < 0.05

Tables (4 and 5) and Fig. 2(a and b) show there was no statistically significant negative correlation between the mean levels of vitamin D and SSS in group I while in group II a positive correlation was found between Vitamin D and SSS (r=0.62, p= 0.001). Nonetheless, the mean vitamin D levels was insignificantly negatively correlated with Modified Rankin scale (mRS) score but in studied group I and was highly significantly negatively correlated with Modified Rankin scale (mRS) score in studied group II. Moreover, in the both studied patient groups, the Modified Rankin scale (mRS) score was highly statistically significantly negatively correlated with the Scandinavian stroke scale (SSS score) .

Table 4: Correlations between different parameters in studied group (I)

Parameters	Patients with ischemic Infarction (Group I) (No =25)					
	mRs		Vit-D		iPTH	
	R =	P -value	R=	P- Value	R=	P -value
Scandinavian stroke scale (SSS)	-0.886**	0.000	0.148	0.479	- 0.122	0.561
Modified Rankin scale (mRS)	-	-	-0.111	0.597	0.131	0.534
Vitamin-D (VIT D)	-0.111	0.597	-	-	-.070	0.739

**Correlation is significant at the 0.01 level (2-tailed)   *. Correlation is significant at the 0.05 level (2-tailed)

Table 5: Correlations between different parameters in studied group (II)

Parameters	Patients with intracerebral Haemorrhage (Group II) (No =25)					
	mRs		Vit-D		iPTH	
	R	P value	R	P Value	R	P value
Scandinavian stroke scale (SSS)	-0.448*	0.025	0.625**	0.001	0.020	0.923
Modified Rankin scale (mRS)	-	-	-0.501*	0.011	-0.033	0.876
Vitamin-D (VIT D)	-0.501*	0.011	-	-	-0.084	0.690

**Correlation is significant at the 0.01 level (2-tailed)   *. Correlation is significant at the 0.05 level (2-tailed)
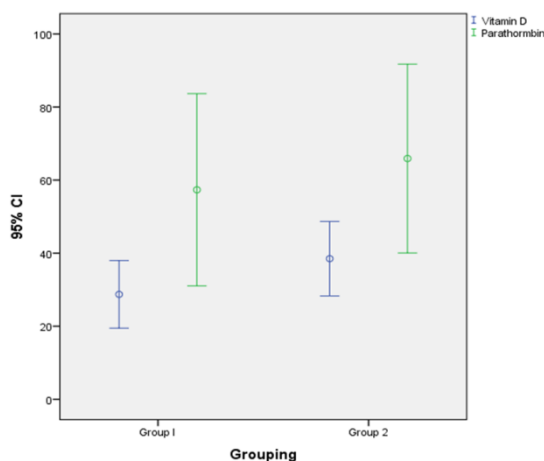
Fig 1

Figure 2(a): correlation between SSS and Vitamin Din group I
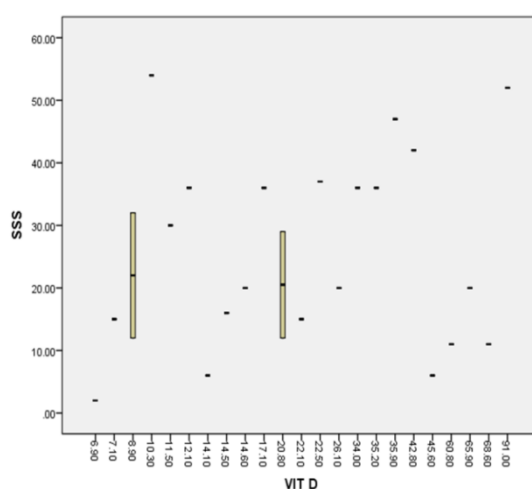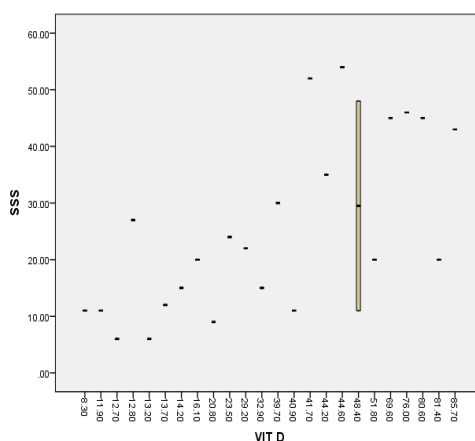

Figure 2 (b) : correlation between SSS and Vitamin D in group II

Using the ROC curve statistical method, we tested our mortality results against the level of Vitamin D and level of iPTH to measure the sensitivity and specificity of these laboratory investigations, and hence detect the method with the best predictability for the mortality in our cases. The area under the ROC curve was measured for each one. Larger values of the test result variable(s) indicate stronger evidence for a positive actual state (better matching). VIT D provided the best results with area under the curve of 0. 64, followed by iPTH with area.
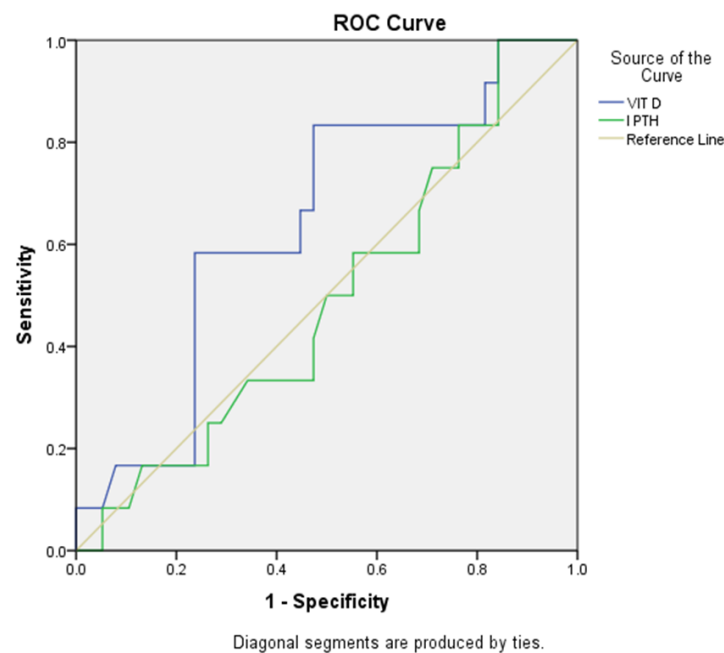
Figure 3: ROC curve of Vit D and iPTH levels

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Discussion

Stroke is one of the leading causes of death and disability worldwide and also increasing trend in low and middle income countries (LMICs)[13]. Vitamin D has a big role in regulation of immunity, cardiovascular and cerebrovascular physiology. Vitamin D has emerged as a potential risk factor for cardiovascular diseases, which has been linked to its vasoprotective potential. The reported vasoprotective effects of vitamin D include slowing down of atherosclerosis, promotion of endothelial function, suppression of renin–angiotensin–aldosterone system and thereby reduction of the risk of hypertension therefore , its deficiency might be involved in the development of several diseases, including arterial hypertension, diabetes mellitus, heart failure, acute myocardial infarction and stroke. Furthermore, vitamin D deficiency influences vascular remodeling through modulation of smooth muscle cell proliferation, inflammation and thrombosis. Also, vitamin D deficiency leads to secondary hyperparathyroidism, and the increase in parathyroid hormone promotes myocyte hypertrophy and vascular remodeling. Parathyroid hormone related protein (PTHrP) acts as a proinflammatory cytokine and may contribute to the instability of the atherosclerotic plaques. These vascular changes are eventually responsible to cause stroke (Thapa et al.,)[14]. Vitamin D deficiency has been associated with many neurological illnesses. This study aimed to assess the status of vitamin D and its association with stroke risk factors and stroke type in patients with acute stroke from Upper Egypt.

The subjects in the present study were relatively younger when compared with stroke patients in similar European and American studies. This could be due to the different population age structure resulting from greater mortality rates in Egypt when compared with more developed countries. This finding was in concordance with V Majumdar et al who reported that the average age of patients with stroke in low-income countries is 15 years below that in high-income countries. Moreover, vitamin D deficiency might bear an association with occurrence of acute stroke in our studied patients whatever its type, and had an association with its prevalent risk factors. Several theories and mechanisms have been explained to describe how vitamin D deficiency can lead to stroke. Our findings was in agreement with Thapa L et al[14] who stated that there was a link between low circulating level of vitamin D and increased

risk of cerebrovascular disease suggesting vitamin D supplementation as a promising approach in the prevention of strokes.

Notably, our current study revealed a highest association between low 25(OH)D levels and increased risk of incident hemorrhagic stroke among people in upper Egypt. This finding could be explained by that deficiency of vitamin D has been associated with hypertension, diabetes mellitus, and hyperlipidemia, which are the known risk factors for hemorrhagic stroke. Pilz et al.[1] who reported an association of low vitamin D levels with cerebrovascular risk factors, in particular arterial hypertension. Moreover, Kojima et al.[15] stated that vitamin D deficiency is associated with several cardiovascular risk factors which could increase the risk of stroke. Kikkinen et al.[16] and Anderson et al.[3] reported that heavy smoking diabetes, hypertension and poor serum lipid profile were associated with low serum vitamin D level. Notably, in our study, most of the patients who had risk factors for stroke such as hypertension, diabetes mellitus, dyslipidemia, smoking, and atrial fibrillation had low levels of vitamin D, suggesting role of vitamin D in these risk factors leading to stroke. Nevertheless, in contrast to our results, Marcénetal.[18] and Kühn etal.[19] had reported no association between vitamin D and cardiovascular diseases. Moreover, Chatterjeeet al.[17] found that vitamin D was lower in non-hypertensive to hypertensive, diabetic to non-diabetic and infarct to intracranial hemorrhage patients, however, none of them were significant.

Hypertension is an established risk factor for stroke. vitamin D has a role in arterial hypertension via the suppression of the renin–angiotensin–aldosterone system (RAS). RAS system is the key regulator of blood pressure, electrolyte and volume homeostasis and its over activation leads to hypertension. Hypertension was the most common modifiable risk factor in our studied patients groups (64%, 76% respectively) and the majority of hypertensive patients (81.25 %) had the more lower levels of

vitamin D suggesting clinical importance. As our results indicate that hypertension mediates the effect of low vitamin D levels on the risk of stroke, in hypertensive subjects, the biological effects of low vitamin D levels are aggravated leading to a pronounced increase in the risk of stroke. Moreover, plasma25-OH-D levels had been inversely associated with risk of incident hypertension in our studied patients, however, with no statistically significant association. Our findings were in agreement with Pokharel etal.[13] who stated that several mechanisms have been proposed on how vitamin D could be involved in blood pressure regulation and the pathophysiology of arterial hypertension, which is a major risk factor for stroke where reported that vitamin D deficiency is associated with increase in renin expression and angiotensin II production, leading to hypertension. Notably, Burgazetal,[20] and Tamez and Thadhani[21] reported an inverse association between vitamin D levels and blood pressure levels as well as the prevalence of arterial hypertension. Also, Scraggetal.[22] were clued that Vitamin D lowers systolic blood pressure . we speculated that the presence of hypertension will aggravate the risk of ischemic stroke associated with low vitamin D levels. Thus, Meticulous management of hypertension, regular monitoring of serum 25 (OH)D levels and treatment of severe vitamin D deficiency, particularly in hypertensive subjects, could help in effective prevention of stroke.

Notably, our study revealed a highest association between low 25(OH)D levels among our stoke patients had type 2 diabetes. This finding could be explained by vitamin D deficiency is related to insulin secretion, insulin resistance, and β-cell dysfunction in the pancreas. Afzal et al.[23] and Song et al.[24] and Nakashima A et al stated Low vitamin D levels have been found to be associated with type 2 diabetes. they explained their results by that the secretion of pancreatic insulin is inhibited by vitamin D deficiency in the diabetic animal model .Moreover, Insulin secretion is also influenced by calcium concentration and flux

through the β-cells. Vitamin D regulates the function of calbindin, a systolic calcium-binding protein found in pancreatic β-cells, and acts as a modulator of depolarization stimulated insulin secretion via regulation of intracellular calcium. PTH, which has its concentration regulated by vitamin D, is associated with insulin synthesis and secretion in the pancreas. Insulin sensitivity is also associated with vitamin D by stimulating the expression of insulin receptors. In addition, vitamin D enhances insulin sensitivity by promoting the expression of peroxisome proliferator-activated receptor (PPAR) delta, which is a widely expressed member of the PPAR family of nuclear receptor fatty acid sensors and regulates fatty acids in skeletal muscle and adipose tissue. Intracellular calcium is a key factor of peripheral insulin resistance via an impaired signal transduction pathway leading to decreased glucose transporter activity. The indirect effect of vitamin D is exerted by regulating calcium flux through the cell membrane and intracellular calcium. While low vitamin D induces secondary hyperparathyroidism, increased PTH levels are also associated with diabetes. Hypovitamin D levels with increased PTH levels were an independent predictor of β-cell dysfunction, insulin resistance, and glycemia.

Moreover, our results revealed the association between low levels of vitamin D and poor lipid profile in the studied acute stroke patients. This finding was in concordance with Kazlauskaite et al.[25] who stated that vitamin D deficiency is associated with large high-density lipoprotein particles. Zittermann et al.[26] stated that vitamin D deficiency has been linked to dyslipidaemia and Ponda et al.[27] observed that low vitamin D concentrations are associated with higher triglycerides and total cholesterol but lower levels of HDL- cholesterol. Nonetheless, Wang et al.[28] showed no significant effect of vitamin D on total cholesterol, HDL-cholesterol or triglycerides, but a marginally significant increase in LDL-cholesterol.

PTH could be considered as a cardiovascular risk factor and higher PTH levels which are a hallmark of vitamin D deficiency, had been considered one of the significant risk factors for cardio-cerebrovascular diseases (van Ballegooijenetal.[29,30] and Pilzetal.[31]). In our study, there was statistically insignificant negative correlation between vitamin D levels and iPTH levels in studied acute stroke patients. This finding was in agreement with Thapaet al.[14] who stated that vitamin D deficiency leads to secondary hyperparathyroidism, and the increase in parathyroid hormone promotes myocyte hypertrophy and vascular remodeling. Notably, Pilzetal.[32] reported the hallmark of vitamin D deficiency is an increase in parathyroid hormone (PTH) levels.

In our study, the studied acute stroke patients, whatever its type, had experienced a severe vitamin D deficiency of less than 50 nmol/L exceeding 84%and 76% in acute ischemic stroke and intracerebral hemorrhage respectively with no significant statistically difference. This is in agreement with Chatterjeeet al.[17] who stated that about 95% of patients that had ischemic infarction and intracerebral hemorrhage had below sufficient levels for vitamin D with the more prevalent insufficient vitamin D levels in patients with ischemic infarction than those with intracerebral hemorrhage. Notably, Park etal.[18]that reported more than 68% of patients with ischemic stroke had vitamin D deficiency (<50 nmol/L). Moreover, Tu et al.[34] and Wang et al.[5] showed 68-78% prevalence of vitamin D deficiency in the Chinese acute ischemic stroke population.

In our current study, there was no association between vitamin D levels and severity of stroke in patients with acute ischemic infarction. Our finding was in consistency with Gupta etal.[6] who stated no association of low vitamin D status with ischemic stroke. Nevertheless, Daubail et al.,[35] and Wang etal.[5] reported that in meta-analysis studies, an increasing risk of ischemic stroke with decreasing concentrations of vitamin D has been observed and stated that the initial neurological deficit is more severe in patients with lower

vitamin D status and vitamin D levels at admission had inversely correlated with the admission neurological deficit (assessed by the NIHSS).

Other studies of Chaudhuri etal.[36], and Sun etal.[4] stated a significant association between vitamin D deficiency and ischemic stroke. Interestingly, Bakradzeetal.[37] reported that Low vitamin D has been found to increase the risk of ischemic stroke, predict severity and poor functional outcome.

Our study demonstrated statistically significant association of low vitamin D levels and severity of stroke in patients with intracerebral hemorrhage. This is in agreement with Bakradzeetal.[37] who found that intracerebral hemorrhage patients with severe vitamin D deficiency had significantly worse stroke severity, where NIHSS on admission was higher in those patients with severe vitamin D deficiency. In spite of these findings, he stated no statistical significance between vitamin D level and discharge mRS score.

Our study, interestingly, experienced no association between vitamin D levels and functional outcome in patients with ischemic stroke. This is incongruity with Park etal.[32] that found an association between the low vitamin D levels and poor functional outcomes in patients with acute ischemic stroke.

Daubailetal.[35] stated that low vitamin D levels was a predictor of functional outcome at discharge and one year mortality in Caucasian stroke population. Similarly, Wang etal.[34] reported that vitamin D level was a predictor of both the severity at admission and the discharge functional outcome in Chinese patients with acute ischemic stroke.

In the current study, our studied stroke patients experienced normal calcium and phosphorus levels .This finding was in contrast to Chatterjee et al.[17] who reported that most of acute stroke patients whatever its types had low serum calcium levels.

Interestingly, in our current study, we found a significant severe deficiency in the level of vitamin D in deaths of our patients with acute stroke whatever its type suggesting that vitamin D could be considered as a prognostic marker of functional outcome and death. This finding was in consistent with Pilzet al.[32] and Zittermann et al.[38] who reported that low vitamin D levels are an independent risk factor for mortality. Notably, Tu et al.[33] statedthat vitamin D is a good marker for prognosis, functional outcome and death in patients with acute ischemic and hemorrhagic stroke. Kikkinenet al.[16] found an inverse association between serum vitamin D level and total cerebrovascular mortality. Previous studies by Giovannucci et al.[39] investigating the link between cardiovascular risk or mortality and vitamin D status had reported low vitamin D levels in these patients.

Carotid atherosclerosis is a progressive multifactorial artery disease associated with high risks of morbidity and mortality (Allison et al.[40]). Carotid intima-media thickness and plaque assessment by cervical ultrasonography is a non-invasive, feasible and accurate method for detecting asymptomatic carotid atherosclerosis (Touboulet al.[41]).

In the current study, abnormal findings in the carotid and vertibrobasilar duplex were seen in our studied acute stroke patients. The most important finding was carotid atherosclerosis. We found that the more prevalence of carotid atherosclerosis has been detected in our acute ischemic stroke patients with high statistically significant difference when compared to patients with intracerebral hemorrhage.

Interestingly, our abnormal findings in the carotid and vertibrobasilar duplex were more prevalent in acute ischemic stroke patients who had lower vitamin D levels. In agreement with our study, Pilz et al.[32] stated that Carotid atherosclerosis leading to increased carotid artery intima media thickness and it is an important risk factor in the development of ischemic stroke, but not with hemorrhagic stroke.

Notably, Giriet al.[42] found a significant negative association between vitamin D and carotid artery intima media thickness in patients with ischemic stroke. Moreover, studies by Carreli et al.[43] have

revealed an inverse association between vitamin D levels and subclinical atherosclerosis as measured by carotid intima-media thickness (IMT) and computed tomography-derived calcified atherosclerotic plaque and this negative association of vitamin D levels with IMT and plaque thickness suggests that vitamin D deficiency may play a role in the development and/or progression of atherosclerosis, which may help to explain the increased risk of CV events and mortality observed in epidemiological studies.

Also, Carreliet al.[43] stated that an association between vitamin D deficiency and carotid vascular abnormalities that predicts the development of myocardial infarction and stroke. However, Blondonet al.[44] and Sachs et al.[45] found no significant association between vitamin D with carotid intima media thickness or carotid plaques.

Conclusion: Vitamin-D insufficiency may bear an association with acute stroke and its prevalent risk factors with a highest association between low 25(OH)D levels and risk of incident hemorrhagic stroke among people in upper Egypt. Low levels of vitamin D are independently predictor for fatal strokes with more evidence of unfavorable outcome in acute intracerebral hemorrhagic stroke, therefore, it might be a marker of greater co-morbidity and a being causally related to stroke suggesting that vitamin D supplementation is a promising approach in the prevention of strokes.

Limitations: Firstly, the sample size was small with no seasonal adjustment for vitamin D levels or assessing its causality to determine the association between vitamin-D level and stroke. Secondly, this study was conducted on Upper Egypt patients and there were several studies have suggested racial differences of association between vitamin D and cardiovascular diseases there. Therefore, further studies are warranted to explore this association among other areas of Egypt and for stroke types. In particular, large-scale clinical trials are needed to guide public health recommendations for lowering the burden of both condition and to shed light on the effects of achieving optimal vitamin D status in the primary prevention of stroke.

References

1. Pilz S, Tomaschitz A, Drechsler C, Zittermann A, Dekker JM, März W. Vitamin D supplementation: a promising approach for the prevention and treatment of strokes. Curr Drug Targets 2011 ;12:88-96

2. Wang L. Song Y, Manson JE, et al. Circulating 25-hydroxyvitamin D and risk of cardiovascular disease: a meta-analysis of prospective studies. Circ Cardiovasc Qual Outcomes. 2012;5:819-829.

3. Anderson JL, May HT, Horne BD,Bair TL, Hall NL,Carlquist JF, Lappé DL, Muhlestein JB. Intermountain Heart Collaborative (IHC) Study Group. Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. Am J Cardiol. 2010;106:963-968.

4. Sun Q, Pan A, Hu FB, Manson JE, Rexrode KM. 25-hydroxyvitamin D levels and risk of stroke : a prospective study and meta-analysis. Stroke 2012;43: 1470-7

5. Wang Y, Ji H, Tong Y etal., (2014) Prognostic value of serum 25-hydroxyvitamin D in patients with stroke. Neurochem Res 39:1332-1337

6. Gupta A, Prabhakar S, Modi M, Bhadada S K,, Lal V, Khurana D. Vitamin D status and risk of ischemic stroke in North Indian patients. Indian Journal of Endocrinology and Metabolism .Sep-Oct 2014; Vol.18, Iss.5:721-5

7. Mitsuhashi T, Morris RC JR, Ives HE (1991) 1,25-Dihydroxyvitamin D3 modulates growth of vascular smooth muscle cells. J Clin Invest 87: 1889-1895.

8. Wang TJ, Pencina MJ, Booth SL,etal. Vitamin D deficiency and risk of

cardiovascular disease. Circulation. 2008;117:503-511.

9. Rigby WF, Denome S, Fanger MW. Regulation of lymphokine production and human T lymphocyte activation by 1,25-dihydroxyvitamin D3. Specific inhibition at the level of messenger rna. J ClinInvest 1987;79:1659-1664.

10. Aihara K, Azuma H, Akaike M, Ikeda Y, Yamashita M, Sudo T et al. Disruption of nuclear vitamin D receptor gene causes enhanced thrombogenecity in mice. J BiolChem 2004; 279: 35798-35802.

11. Michos ED, and Gottesman RF. Vitamin D for the prevention of stroke incidence and disability: Promising but too early for prime-time. Eur J Neurol. 2013 January; 20 (1):3-4.

12. Witham MD, Dove FJ, Sugden JA, Doney AS, Struthers AD. The effect of vitamin D replacement on markers of vascular health in stroke patients- a randomized contro-lled trial. Nutr Metab Cardiovasc Dis 2012;22:864-70.

13. Pokharel BR, Kharel G, Thapa LJ, Rana PVS. Vitamin D and other risk factors among stroke patients. Kathmandu Univ Med J 2015;49(1):71-3

14. Thapa L, Shrestha A, Pradhan M, Bhandari T R, Shrestha S, Poudel RS, Pokhrel B, Poudel R. Status of vitamin D and its association with stroke risk factors in patients with acute ischemic stroke in a tertiary carte hospital. J Nepal Med Assoc 2014;52(195):935-39.

15. Kojima G, Bell C, Abbott RD, Launer L, Chen R, Motonaga H, etal.Low dietary vitamin D predicts 34-year incident stroke: the Honolulu Heart Program. Stroke 2012; 43:2163-7

16. Kikkinen A, Knert P, Aro A, Rissanen H, Marniemi J, Heliövaara M, Impivaara O, Reunanen A. Vitamin D status and the risk of cardiovascular disease death. Am J Epidemiol 2009;170:1032-1039.

17. Chatterjee K, Mandal SK, Chatterjee S, Sarkar P, Mondal SS, Ganguly J, Sardar D, Mani S, Das B. Assesment of vitamin D in cerebrovascular accident patients in Eastern India. National Journal of Medical Research , vol.4,iss. 4, Oct-Dec 2014;264-269

18. Marcén R, Jimenez S, Fernández-Rodriguez A,Galeano C, Villafruela JJ, Gomis A, etal. Are low levels of 25-hydroxyvitamin D a risk factor for cardiovascular disease or malignancies in renal transplantation. Nephrol Dial Transplant 2012 ; 27(Suppl 4): iv47-52

19. Kühn T, Kaaks R, Teucher B, Hirche F, Dierkes J, Weikert C, Katzke V, Boeing H, Stangl GI, Buijsse B (2013) Plasma 25-hydroxyvitamin D and its genetic determinants in relation to incident myocardial infarction and stroke in the European prospective investigation into cancer and nutrition (EPIC)- Germany study. PloS ONE 8:e69080.

20. Burgaz A, Orsini N, Larson SC,Wolk A (2011) Bood 25-hydroxyvitamin D concenteration and hypertension: a meta-analysis. J Hypertens29:636-645

21. Tamez H and Thadhani RI. Vitamin D and hypertension: an update and review. CurrOpin Nephrol Hypertens 2012; 21:492-9

22. Scragg R, Wishart J, Stewart A, Ofanoa M, Kerse N, Dyall L, etal. No effect of ultraviolet radiation on blood pressure and other cardiovascular risk factors . J Hypertens 2011;29:1749.

23. Afzal S, Bojesen SE, Nordestgaard BG. Low 25-hydroxyvitamin D and risk of type 2 diabetes: a prospective cohort study and meta-analysis. Clin Chem. 2013;59:381-391.

24. Song Y, Wang L, Pittas AG, Del Gobbo LC, Zhang C, Manson JE, Hu FB (2013) Blood 25-hydroxyvitamin D levels and incident type 2 diabetes: a meta-anlysis of

prospective studies. Diabetes Care 36: 1422-1428.

25. Kazlauskaite R, Powell LH, Mandapakal C, Cursio JF, Avery EF,Calvin J. Vitamin D is associated with atheroprotective high-density lipoprotein profile in postmenopausal women. J ClinLipidol 2010;4:113-119.

26. Zittermann A, Gummert JF, Börgermann J. The role of vitamin D in dyslipidemia and cardiovascular disease.Curr Pharm Des 2011; 17: 933-42.

27. Ponda MP, Huang X,Odeh MA, Breslow JL, Kaufman HW (2012) Vitamin D may not improve lipid levels: a serial clinical laboratory data study. Circulation 126: 270-277.

28. Wang H, Xia N, Yang Y, Peng DQ (2012) Influence of vitamin D supplementation on plasma lipid profile: a meta-analysis of randomized controlled trials. Lipids Health Dis 11:42.

29. van BallegooijenAJ,ReindersI,Visser M, Dekker JM, Nijpels G, Stehouwer CD, Pilz S, Brouwer IA (2013) Serum parathyroid hormone in relation to all-cause and cardiovascular mortality: the Hoorn study. J ClinEndocinolMetab 98: E638-E645.

30. vanBallegooijenAJ,ReindersI,Visser M, Brouwer IA (2013) Parathyroid hormone and cardiovascular disease events: a systematic review and meta-analysis of prospective studies. Am Heart J 165: 655-664.

31. Pilz S, Tomaschitz A, Drechsler C, Dekker JM, März W. Vitamin D deficiency and myocardial diseases. MolNutr Food Res 2010; 54 :1103-13

32. Pilz S, Gaksch M., O'Hartaigh B, Tomaschitz A, März W. The role of vitamin D deficiency in cardiovascular disease: where do we stand in 2013? Arch Toxicol (2013) 87: 2083-2103.

33. Park K Y, Chung PW, Kim YB, Moon HS, Suh BC, Won YS, Kim JM, Youn YC, Kwon OS . Serum vitamin D status as a predictor of prognosis in patients with acute ischemic stroke.Cerebrovasc Dis 2015;40:73-80

34. TuWJ,ZhaoSJ,Xu DJ etal (2014) Serum 25-hydroxyvitamin D predicts the short-term outcomes of Chinese patients with acute ischemic stroke. ClinSci 126(5) :339-346

35. Daubail B, Jacquin A, Guiland JC, Khoumri C, Aboa-Eboulé C, Giroud M, Béjot Y: Association between serum concentration of vitamin D and 1-year mortality in stroke patients. Cerebrovasc Dis 2014 ; 37:364-367

36. Chaudhuri JR, Mridula KR, Alladi S, Anamika A, Umamahesh M, Balaraju B, Swath A, Bandaru VS. Serum 25-hydroxyvitamin D deficiency in ischemic stroke and subtypes in Indian patients. J Stroke 2014; 16:44-50

37. Bakradze E, McCullough L, Staff I, Nouh A (2016)Vitamin D deficiency correlates with stroke severity on presentation in intracerebral hemorrhage. Stroke; Feb., Vol.47,Iss. Supp 1

38. Zittermann A, Iodice S, Pilz S, Grant WB, Bagnardi V, Gandini S (2012). Vitamin D deificiency and mortality risk in the general population: a meta- analysis of prospective cohort studies. Am J ClinNutr 95:91-100..

39. GiovannucciE,LiuY,Hollis BW, Rinm EB. 25-hydroxyvitamin D and risk of myocardial infarction in men. Arch Intern Med. 2008:168:1174-1180.

40. Allison M, His S, Wassel C, Morgan C, Joachin H, Wright C, etal. Calcified atherosclerosis in different vascular beds and the risk of mortality. Arterioscler ThrombVascBiol, 2012 ; 32:140-6

41. Touboul PJ, Grobbee DE, RuijterHD. Assesment of subclinical atherosclerosis by carotid intima media thickness:

technical issues. European Journal of preventive Cardiology, 2012;19(2):8-24

42. Giri R, Rai R, Verma S, Verma RK .Correlation between vitamin D and carotid artery intima media thickness in patients with ischemic stroke. Iternational J of Advances in Medicine ,April-june 2016, Vol 3,Issue 2;398-393.

43. Carreli A L, Walker M D, Lowe H, McMahon D J, Rundek T, Sacco RL and Silverberg S J. Vitamin D deficiency is associated with subclinical carotid atherosclerosis: The Northern Manhatten study . Stroke, 2011;42:2240-2245.

44. Blondon M, Sachs M, Hoofnagle AN, TX JH, Michos ED, KorcarzC,etal. 25-hydroxyvitamin D and parathyroid hormone are not associated with carotid intima- media thickness. Arteioscler Thromb VascBiol, 2013;33 (11):2639-45.

45. Sachs MC, Brunzell JD , Cleary PA, Hoofnagle AN, Lachin JM, Molitch ME, Steffes MW, Zinman B, de Boer IH. Diabetes control and complication Tria/Epidemiology of Diabetes Interven-sions and Complications Study (DCCT/EDIC) Research Group (2013) Circulating vitamin D metabolites and subclinical atherosclerosis in type l diabetes. Diabetes Care 36:2423-2429.

46. Akio Nakashima, Keitaro Yokoyama, Takashi Yokoo, Mitsuyoshi Urashima : Role of vitamin D in diabetes mellitus and chronic kidney disease. *World J Diabetes* 2016 March 10; 7(5): 89-100.

47. V Majumdar, P Prabhakar, GB Kulkarni and R Christopher : Vitamin D status, hypertension and ischemic stroke: a clinical perspective. Journal of Human Hypertension (2015) 29, 669–674.