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Development and estimation methods of 25-hydroxyvitamin D

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***Abstract*---**Stroke is a worldwide health problem. It is one of the most common and most devastating disorder of central nervous system. Not only the mortality associated with stroke, but also the morbidity puts a heavy emotional, psychological and financial burden both on the patient as well as on the family and the state. To study serum level of 25-hydroxyvitamin D in acute stroke patients and to evaluate any association between 25(OH) vitamin D level and stroke and its risk factors. The present study had been conducted at VIMSAR from September 2013 to September 2015. For this study 73 patients with acute stroke were taken as cases and 40 subjects were taken as controls. In our study cases are having professions in which they get adequate exposure to sun rays like farmers and daily labours. They have been found to be having significantly higher level of serum vitamin D (42.81) as compared to the other group (26.52) with a p value of 0.01.

***Keywords*---**Serum, Vitamin D, Hemorrhagic Stroke, Emotional, Psychological.

1. Introduction

In 2013, stroke was the second most frequent cause of death after coronary artery disease, accounting for 6.4 million deaths (12% of the total). About 3.3 million deaths resulted from ischemic stroke while3.2 million deaths resulted from haemorrhagic stroke. (1) Stroke is a worldwide health problem. It is one of the most common and most devastating disorder of central nervous system. Not only the mortality associated with stroke, but also the morbidity puts a heavy emotional, psychological and financial burden both on the patient as well as on the family and the state. Stroke is one of the leading causes of death and disability in india. Prevalence of stoke in india varies from 84-262 per 100000 in rural India and 334-424 per 100000 population in urban India. Incidence rate is 119-145 per 100000 population based on recent population studies. (2) Recent data, taking account of stroke survivors and stroke caused deaths, suggest higher stroke rates as compared to the developed nations; the prevalence rate being 545.10 per 100,000 (95% CI,479.68 to 617.05) and annual incidence rate of first ever stroke at *145.30(95%* CI ,120.39 to 174.74); overall 30 days case fatality being 41 .08%. (3)

Stroke is defined as rapidly developing clinical signs of focal or global disturbance of cerebral function with symptoms lasting 24 hours or longer or leading to death with no apparent cause other than vascular origin. By this definition TIA which is defined to last less than 24 hours and patient with stroke symptoms caused due to trauma, subdural haemorrhage, tumours or poisoning are excluded. (4) There are many risk factors for stroke including age, sex, family history of stroke, hypertension, smoking, diabetes, obesity, hyperlipidemia and atrial fibrillation. Many studies indicate a plethora of conventional risk factors for stroke.(5)

Nevertheless, cerebrovascular events do occur sometimes in the individuals without any of the previously mentioned risk factors. As a consequence, it is very likely that other risk factors exist. Identification of modifiable risk factors for stroke may lead to more effective prevention of first and recurrent episodes of cerebrovascular disease. Data from prospective observational studies indicate that vitamin D deficiency is an independent risk factor for stroke. (5,6).

The major and most well-known function of vitamin D is to maintain calcium and phosphorus homeostasis and bone mineralization. However recent evidences suggest that vitamin D may be important for a variety of no skeletal outcome. Since 25(OH)D can cross blood brain barrier and vitamin D receptor have been identified within brain, it has been suggested that vitamin D may exert neuroprotective actions. More detailed evaluations showed that vitamin D deficiency caused a dysregulation in the inflammatory response and decreased neuroprotective factors such as insulin growth factor-1. In experimental studies VDR activation has been shown to exert a variety of antiatherosclerosis effects. (7)

2. Aims and Objectives

To study serum level of 25-hydroxyvitamin D in acute stroke patients and to evaluate any association between 25(OH)vitamin D level and stroke and its risk factors**.** It is an observational study. It includes 73 cases and 40 controls. After

hospitalization ,a detailed history was taken from all cases and controls including history of lifestyle and dietary habit and a thorough physical examination was performed so as to fulfill the inclusion and exclusion criteria laid down in the study protocol.

3. Material & Method

3.1 Place and Period of Study:
The Department of MEDICINE, VIMSAR, BURLA, SAMBALPUR from September 2013 – 2015.

3.2 Case:
73 patients, diagnosed as acute stroke from history and clinical findings and confirmed by radiological investigations (CT/MRI) were taken as cases.

3.3 Control:
40 age and sex matched persons were taken as controls who were not having stroke or any risk factor for it.

3.4 Ethics statement and informed consent
This study was conducted according to the principle expressed in the Declaration of Helsinki and approved by the ethics committees of the Medical College as well as the ethics committees of Sambalpur University. Written informed consent was obtained in prescribed format from parents or guardians of all study patients.

3.5 Inclusion Criteria
Patients with acute stroke were included in this study. They were subjected to thorough clinical examination and evaluated for risk factors like hypertension, diabetes mellitus, hyperlipidaemia, lifestyle, dietary habbit, etc.

3.6 Investigation to be done:
1. Routine haematologically examination
2. Renal parameters
3. LFT
4. CT scan of brain
5. Fasting blood sugar level
6. Lipid profile
7. Serum 25-hydroxyvitamin D level
8. ECG
9. ECHO if necessary

3.7 Exclusion criteria
Chronic kidney and liver disease
Pregnancy
Persons taking vitamin D supplements

4. Methods of 25-Hydroxyvitamin D Estimation

4.1 Methods of Study

This study was conducted taking a sample of 73 patients of acute stroke and 40 age and sex matched normal subjects from different sources of the hospital with no risk factors for stroke and had no history of any cerebro vascular disease. Then serum 25-hydroxyvitamin D was estimated in both subjects and made a comparision between the two.

PROFORMA FOR PATIENTS WITH STROKE

NAME- UNIT-MMW/FMW-I/II/III/IV/V/VI
BED NO-
FATHER/HUSBAND'S NAME- DATE OF ADMISSION-
IRN-
AGE- SEX:M/F DATE OF DISCHARGE/DEATH-
OCCUPATION-
CONTACT NO-
ADDRESS-
DIAGNOSIS-
PRESENTING FEATURE:
HEMIPLEGIA/MONOPLEGIA(RT/LT/UL/LL)
LOSS OF CONSCIOUSNESS
CON VULSIONS (FOCAL/GENERALISED)
HEADACHE/VOMITING/VERTIGO/DIZZINESS
LOSS OF SPEECH
UNSTEADINESS OF GAIT/VISUAL BLURRING/DIPLOPIA/BURNING PAIN(RT/LT/UL/LL)
INCONTINENCE OF URINE/FACIAL WEAKNESS
MENTION IF ANY
CIRCUMSTANCE DURING ONSET-AWAKE/RESTING/ACTIVITY/SLEEP
PAST HISTORY: DISEASE/DURATION/TREATMENT
RECEIVING/HYPERTENSION/DIABETES/
DYSLIPIDEMIA/CKD/STROKE
STROKE-1st-ISCHAEMIC/HAEMORRHAGIC/SITE/SIDE/NEUROLOGICAL DEFICIT
2nd-ISCHAEMIC/HAEMORRHAGIC/SITE/SIDE/NEUROLOGICAL DEFICIT
ARRHYTHMIAS-ATRIAL FIBRILLATION OR ANY OTHER/
MYOCARDIAL INFARCTION-DATE/TIME
CONGENITAL HEART DISEASE IF ANY:ASD/VSD/PDA/TOF/MVP
VALVULAR HEART DISEASE IF ANY:MS/MR/AS/AR/TR/PR/BAV
CARDIOMYOPATHY: DCM/RCM/HOCM IF ANY
PERSONAL HISTORY:VEGETARIAN/ NON-VEGETARIAN/ MIXED/ADDICTION IF ANY/ LIFESTYLE
CONTACT HISTORY: YES/NO, SOCIOECONOMIC STATUS: POOR/AVERAGE/GOOD
TREATEMENT HISTORY: NAME OF THE DRUGS, DOSES AND DURATION OF TREATEMENT

GENERAL EXAMINATION- PULSE - BP- TEMP.-
PALLOR - ICTERUS-
CYANOSIS - CLUBBING - -
OEDEMA - LYMPHNODES -
RESP. RATE-

CHEST-ABNORMALITIES IF ANY
CVS -APEX S1 S2 S3 S4 MURMUR- RUB
P/A -
CNS-HIGHER FUNCTIONS
CRANIAL NERVES
MOTOR SYSTEM
SENSORY SYSTEM
AUTONOMIC SYSTEM
CEREBELLAR SIGNS
INVESTIGATION:
1)HAEMATOLOGICAL PROFILE-HB% DC- TLC
ESR
BT CT SICKLING

2) BIOCHEMICAL - FBS SR.CREATININE
BLOOD UREA SR.ALBUMIN SR. 25-HYDROYVITAMIN D FASTING
LIPID PROFILE
3)IMMUNOLOGICAL PROFILE- HIV HBV HCV.
ECG :
ECHOCARDIOGRAPHY:
CT SCAN BRAIN :
TREATMENT PROVIDED-

ACEI ANTIPLATELET
FOLIC ACID
ARB BETA BLOCKER
STATINS ANTIDIABETIC DRUGS
FIBRATES OTHER T/T IF ANY
COURSE DURING HOSPITAL STAY:
CONDITION ON DISCHARGE :
FOLLOW UP OF THE PATIENT:

The present study had been conducted at VIMSAR from September 2013 to September 2015. For this study 73 patients with acute stroke were taken as cases and 40 subjects were taken as controls.

5. Experimental Methods

5.1 DEMOGRAPHIC STUDY (AGE, GENDER & PROFESSION)

Table-1 Age Distribution among Case and Control

Age Distribution	CASES	CONTROLS
30-39yrs	4(5.4%)	3(7.5%)

40-49yrs	15(20.5%)	7(17%)
50-59yrs	14(19.1%)	5(10%)
60-69yrs	26(35.6%)	17(42%)
70-79yrs	9(12.3%)	6(15%)
>80yrs	5(6.8%)	2(5%)
Total	73	40

Our study maximum 35.6% cases were from 60-69 yrs age group & almost 87 % belongs to 40-80 yrs age group which is comparable to the control group where 84% belongs to 40-80 yrs

Table 2 Age and sex profile among case and control

	CASES	CONTROL
AGE(Median)	63Yrs	60 Yrs
SEX(Male : Female)	38:35(n=73)	24:16(n=40)

The median of age among cases is 63yrs where as that of controls is 60 yrs. Among the cases there are 38 males & 35 females, in controls there are 24 males &16females.

Table 3 Presenting features in acute stroke patients

Presenting features	No of patients	Percentage
Hemiplegia	61	83.56%
Loss of consiousness	29	39.73%
Dysphasia	12	16.43%
Convulsion	3	4.10%
Others(Vertigo, vomiting, ataxia etc)	4	5.47%

As usual weakness of one half of body was the commonest complaint61(83.56%). Diminished level of consciousness was a features at presentation in 29(39.73%) cases, whereas dysphasia and convulsion was present at admission in 16.43% cases and 4.10% respectively.

Table – 4 Comparison of level of vitamin–D among male & female

		SUBJECTS	Mean (ng/ml)	SD	P value
CASES	MALE	38	22.95	8.60	0.50
	FEMALES	35	21.62	8.10	
CONTROLS	MALE	24	27.66	5.01	0.001
	FEMALES	16	22.14	2.67	

The comparison of level of Vitamin D with gender is found to be significant in controls with a p value of 0.001 where as it is not significant in cases. In our study, males are found to be having higher vitamin D level as compared to females

Table – 5 Professional demography study

PROFESSION	SUBJECTS	Mean VIT-D (ng/ml)	SD	SEM	P value
Adequate exposure	11	42.81	20.20	6.41	0.01
In- adequate exposure	27	26.52	12.81	2.50	

In our study 11 cases are having professions in which they get adequate exposure to sun rays like farmers and daily labours. They have been found to be having significantly higher level of serum vitamin D (42.81) as compared to the other group (26.52) with a p value of 0.01.

Table – 6 Comparison Of Level Of Vitamin D Among Cases & Controls Case & Control Comparison

Group		SUBJECTS	Mean OF VIT – D (ng/ml)	
Group	Category	N	Mean 25(OH) VIT D level (ng/ml±SE)	Significance level in comparison to control
1	Control	40	26.43±0.71	-
2	Haemorrhagic stroke	33	26.12±1.55	P= 0.84
3	Ischemic stroke	40	18.30±0.85	P=0.000

The mean 25(OH) vitamin D is significantly low in ischemic stroke in comparison to control ($p<0.001$), but not in haemorrhagic stroke.

In our study 11 cases are having professions in which they get adequate exposure to sun rays like farmers and daily labours. They have been found to be having significantly higher level of serum vitamin D (42.81) as compared to the other group (26.52) with a p value of 0.01.

6. Conclusion

Our study maximum 35.6% cases were from 60-69 yrs age group & almost 87 % belongs to 40-80 yrs age group which is comparable to the control group where 84% belongs to 40-80 yrs The median of age among cases is 63yrs where as that of controls is 60 yrs. Among the cases there are 38 males & 35 females, in controls there are 24 males &16 females. As usual weakness of one half of body was the commonest complaint61(83.56%). Diminished level of consciousness was a features at presentation in 29(39.73%) cases, whereas dysphasia and convulsion was present at admission in 16.43% cases and 4.10% respectively In our study 11 cases are having professions in which they get adequate exposure to sun rays like farmers and daily labours. They have been found to be having significantly higher level of serum vitamin D (42.81) as compared to the other group (26.52) with a p value of 0.01.

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