"Impact of genes polymorphism and vitamin supplementations in hyperhomocysteinemia among ischemic stroke patients with a view to develop a self- instructional discharge module" A study protocol.

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Abstract

Introduction: Stroke is a leading cause of long-term disability and the leading preventable cause of disability. Among ischemic stroke patients, severe hyperhomocysteinemia is due to rare genetic defects resulting in impairment of the methylation pathway like deficiencies of folate or Vitamin B_{12} or CBS, MTHFR thermolability.

Aim: To assess the impact of genetic polymorphisms and vitamin B_6, B_{12}, Folic acid supplementations on homocysteine level.

Materials and Methods: A quantitative, analytical-observational research design will be used for the study protocol. Samples will be the ischemic stroke patients who come to emergency room, Neuro OPD and Neuro ward at AIIMS, Rishikesh, Uttarakhand selected by using purposive sampling technique. Sample size will be calculated after pilot study.

Tools: Subject Datasheet, NIH Stroke Scale (11items) and MRS (7items) will be used for the study. Testing for genetic polymorphisms in homocysteine, serum vitamin-B_{12}, folic acid and serum homocysteine level will be done.

Follow-up:-Total 15 months (at 1, 3, 9 & 15 months).

Module development and its validation:

At the end of data collection process, self-instructional discharge module will be developed and validated by experts from the items related to module.

Statistical analysis: The data will be collected and analyzed by using appropriate descriptive and inferential statistics at p value considered to be significant <0.05.

Discussion: This study will become beneficial for the ischemic stroke patient by decreasing level of homocysteine and risk of re-occurrence of cardiovascular related disorders.

Keywords: *Ischemic stroke patients, hyperhomocysteinemia, genetic polymorphisms, vitamin supplementations, self-instructional discharge module.*

Introduction-

According to World Health Organization (WHO) 2015, "Stroke is defined as a condition caused by the interruption of the blood supply to the brain, usually because a blood vessel bursts or is blocked by a clot [(1)]. "There are three main kinds of stroke, Transmic ischemic attack (TIA), ischemic stroke and hemorrhagic stroke. Ischemic stroke is the most common form of stroke, accounting for around 85% of strokes." [(2)].

Stroke is at fifth number for causing death in the United States, killing around 130,000 people in a year. Someone in the United States has a stroke every 40 seconds.

The estimated adjusted prevalence rate of stroke range, 84-262/100,000 in rural and 334-424/100,000 in urban areas in India.

The incidence rate is 119-145/100,000 based on the recent population based studies. There is also a wide variation in case fatality rates with the highest being 42% in Kolkata. Even though India is a leading generic drugs producer still many people can't afford the commonly used secondary prevention drugs [3].

Among Sub-Saharan African country 90-day mortality of patients presenting of stroke at MNH hospital is around 50% [4]. After 15 months of follow-up with sustained hyperhomocysteinemia reoccurrence of the new cerebrovascular events were classified as cerebral infarction in 57.1% [5].

Homocysteine is a sulfur amino acid whose metabolism stands at the intersection of two pathways: remethylation to methionine, which requires folate and vitamin B_{12} and transsulfuration to cystathionine, which requires pyridoxal-5'-phosphate. Severe hyperhomocysteinemia is due to rare genetic defects resulting in impairment of the methylation pathway like deficiencies of folate or Vitamin $_{B12}$ or CBS, MTHFR thermolability. Post-methionine-load hyperhomocysteinemia may be due to heterozygous CBS gene defect or B6 deficiency [6]. Normal homocysteine concentrations are from 5 to 15 µmol/l.

Elevated total homocysteine level is found to be an independent risk factor for recurrent stroke. In 2002, Wald et al. conducted meta-analysis where lowering homocysteine by 3 µmol/L was reported to reduce the risk of ischemic heart disease by 16%, deep vein thrombosis by 25% and stroke by 24% [7].

Blood sample for homocysteine will be tested with help of chemiluminescent immunoassay technique at biochemistry laboratory. Immunoassay methods is the measure by concentration of analytes through antigen–antibody reactions utilizing at least one reagent which is an antibody specific to the analyte. Depending on specific method protocols, immunoassays can be performed in a timeframe of a few hours or up to two days [8].

Most common genetic polymorphisms found in homocysteine metabolism is in SNPs of MHTFR-C677T, MS-D919G and CBS-1278T [9].

Genetic polymorphisms are defined as the occurrence of multiple allele at a locus, where at least two alleles occur with a frequency greater than 1%. Genetic polymorphisms is tested by PCR-RFLP method, RFLP is a technique invented in 1984 by the English scientist Alec Jeffreys during research into hereditary diseases. It works by using following steps:- DNA extraction, DNA Fragmentation, gel electrophoresis and visualization of bands [10].

Role of vitamin B6, B12 and folic acid is found to be effective in reducing homocysteine level among stroke patients. Vitamin B6 is a co-enzyme for amino acid metabolism. Normal serum Vitamin B6 level is 30-80 ng/ml among adults [11]. Vitamin B12 requires for RBC formation, neurological functions and DNA synthesis. Normal Serum vitamin B12 level in adults is 170-200pg/ml [12]. Folic acid is absorbed two-folds better than folates. Normal plasma folate level in adults is 2-20ng/ml [13].

Need and scope of the study:-

There is a lack of existing data on MS, CBS genetic polymorphisms involving homocysteine metabolic pathway but MTHFR polymorphisms is well known among ischemic stroke patients.

Although neurologists are prescribing vitamin B supplementations to ischemic stroke patients for better recovery. It will help to improve patient recovery and quality of life by reducing mortality and morbidity from reoccurrence of stroke.

It will reduce hospital burden by decreasing re-hospitalisation. However, no observational study has been found in North India.

Thus, this data would be needed which help in decreasing homocysteine level, preventing re-occurrence of stroke among ischemic stroke patients.

Operational definitions:-

Ischemic stroke: -

Patient who come to emergency department reported symptoms of hemiparesis/hemi-paralysis, slurred speech, blurred vision for the first time within 72 hours of occurrence of ischemic stroke which will be confirmed by CT/MRI report.

Genetic polymorphisms: -

Any polymorphism found in SNPs of MTHFR-C677T, MS- D919G, CBS-1278T which will interfere in the homocysteine metabolic pathway.

Hyperhomocysteinemia:-

Normal serum homocysteine level=3.7-13.9µmol/L assessed with help of chemiluminescent immunoassay technique Hyperhomocysteinemia>15µmol/L

Vitamin supplementations: -

Normal Vitamin B_{12} (serum, Heparinized) = 211-911pg/ml and folate (folic acid) = 11.1-79.5pg/ml assessed with help of chemiluminescent immunoassay technique. In this study tablet Homocheck, tablet Folvite will be prescribed to participants BD for six months by neurologists.

Assumptions:-

Ischemic stroke patients were having MTHFR, MS, CBS genes polymorphisms which causes interruption in homocysteine metabolism pathway led to hyperhomocysteinemia and ischemic stroke condition.

Ischemic stroke patients were having deficiency of vitamin B_6, B_{12} and folic acid which led to interruption in homocysteine metabolism pathway led to hyperhomocysteinemia and ischemic stroke condition.

The patients will be honest in giving answers to the items in the tools.

Delimitation: - The study is delimited to clinically stable patients with ischemic stroke, who are coming to the emergency department, neurology OPD and neurology ward, AIIMS, Rishikesh.

Aims & Objectives: -

Primary Objectives-

1. To assess the impact of genetic polymorphisms on homocysteine level.
2. To assess the effect of vitamin B_6, B_{12}, Folic acid supplementations on homocysteine level.

Secondary Objectives-

1. To assess the reoccurrence of stroke or any major cardiovascular events
2. To find out association between MTHFR, MS, CBS genes polymorphism and risk of recurrent ischemic stroke.
3. To develop and validate a self-instructional discharge module.

Material and Methods:

Research approach-A quantitative research approach use for the study. **Research Design-**Analytical-observational research design use for the study. **Sample-** Sample of the study are ischemic stroke patients who come in emergency room, Neuro Outpatient department and Neuro ward at AIIMS, Rishikesh, Uttarakhand, India. **Sample Size-**Sample size will be calculated after pilot study. **Sampling Technique-**Purposive sampling technique use for the study.

Inclusion criteria:-

Patients who are in the age group of 18-70 years, having history of Ischemic stroke within 72 hours and confirmed by CT/MRI report, having hyperhomocysteinemia confirmed by blood test report, prescribed with vitamin B6, B12 and folic acid supplementation, having normal renal function confirmed by KFT report, able to communicate in English and Hindi language, who given consent to participate in the study.

Exclusion criteria:-

Patients suffering from neurological conditions other than Ischemic stroke such as Migraine, Alzheimer dementia, Parkinson's disease, having recurrent Ischemic stroke and having significant disabilities, who are having stroke due to pregnancy, complication of any surgery and history of hypothyroidism, psoriasis, malignancies, Rheumatoid disease were excluded from the study.

Patients who having history of vitamin B6, B12, Folic acid supplementations, sex hormonal preparations like androgens intake from six months prior to attack of Ischemic stroke and were prescribed with drugs like methotaxate, cyclosporine, theophylline, anticonvulsants like phenytoin, carbamazepine, lipid lowering like cholestyramine, nicotinic acid derivatives (e.g. fenofibrate) excluded from the study.

Tools and methods of data collection:-

- **Subject Datasheet** including socio-demographic and Clinical variables
- **Tool no.1-** NIH Stroke Scale (11)
- **Tool no.2-** MRS (7)
- **Investigations-** Serum homocysteine level, Vitamin B12, folic acid, genetic testing for MTHFR, MS, CBS genes polymorphism.

Description of tools:-

Subject datasheet includes socio-demographic variables such as subject's age, gender, marital status, education, occupation, per month family income, habitat and number of family members. Clinical variables such as BMI, hypertension, diabetes mellitus, history of heart disease, any other illness, homocysteine level, type of ischemic stroke, duration of stroke, type of substance abuse, pattern of substance abuse, frequency of substance abuse, number of cigarettes/bidi/tobacco chews per day, amount of alcohol use per day, prescribed with drugs like antiplatelet, antihypertensive, anticoagulants, statins, antacids, analgesics, antipyretics, antibiotics, anticonvulsants. **Tool no. 1. NIH Stroke Scale-**The National Institutes of Health Stroke Scale, or NIH Stroke Scale (NIHSS) is a standardized tool to check stroke severity. NIHSS is composed of 11 items, each of which scores a specific ability between 0 and 4. For each item, a score of 0 indicates normal function in that specific ability, score 1-4 showing minor stroke, 5-15 moderate stroke, 16-20 moderate to severe stroke while a higher score 21-42 is indicative of some level of impairment. The maximum possible score is 42, with the minimum score being 0. Correlation coefficient or κ coefficient is 0.92. Competency certificate for using this tool has been taken freely online. **Tool no. 2. MRS-** Modified Rankin Scale (MRS) is a standardized tool by Rankin J et al, 1957. It measures degree of disability among stroke patients and includes seven items with score ranges from 0-6. Minimum score "0" indicates no symptoms at all and maximum possible score "6" indicates dead. Intraclass correlation coefficient is 0.94.

Steps of data collection process:-

1. Subjects will be recruited in the study according to inclusion and exclusion criteria with use of purposive sampling technique.
2. Subjects will be enrolled by taking willingness via participant information sheet and participant informed consent form.
3. Socio-demographic details of subjects will be filled by researcher.
4. Venous blood sample of 5-10 ml for Vitamin B_{12}, Folic acid, homocysteine (via chemiluminescent Immunoassay) and MTHFR-C677T, MS-D919G, CBS-1278T genes polymorphisms (via PCR-RFLP technique) involved in homocysteine metabolism will be taken from subjects at the time of recruitment as baseline value.
5. All genetic testing will be done by researcher as she had taken training to conduct DNA analysis via PCR-RFLP technique independently already taken for two and half months (15 Febuary-30 April 2019) from AIIMS, New Delhi, India.
6. For assessing stroke severity, NIHSS and for global disability, MRS tool will be administered by researcher.
7. Oral supplementations in form of oral tablets/capsules containing composition of vitamin B6, B12 and folic acid supplementation in form of (Tab. Homocheck, Tab Folvite) BD for 6 months will be prescribed by neurologists to all recruited ischemic stroke patients.
8. On follow-up visit of total fifteen months at 1, 3, 9 & 15 months subject's serum homocysteine levels will be assessed by withdrawing 5ml of blood at each visit and NIHSS, MRS will be administered at each hospital visit.

Follow-up:- This study included follow-up upto fifteen months (0, 1, 3, 9 & 15 months). At the end of the data collection, **a self-instructional discharge module** (contains diet, drug & vitamin supplementations, physiotherapy/exercises, early warning signs, physical care, psychological care and tips for caregivers to keep them healthy) will be developed and getting validated by related experts.

PLEASE PUT IMAGE HERE GIVEN AT THE END.

Potential benefits of intervention: - No intervention

Potential risks / side-effect due to intervention:- NA

Ethical clearance

Ethical clearance will be obtained from the Institutional Ethics Committee of AIIMS, Rishikesh. Informed written consent and confidentiality of information and anonymity will be maintained. Permission to use the standardized tools will be taken from copyright authors.

Pilot study

The pilot study will be conducted for assessing the feasibility of the study.

Statistical Analysis

The data will be collected, coded and summarized in Excel master data sheet and analyzed based on the objectives of the study using SPSS software. Appropriate descriptive and inferential statistics will be used to analyze the data.

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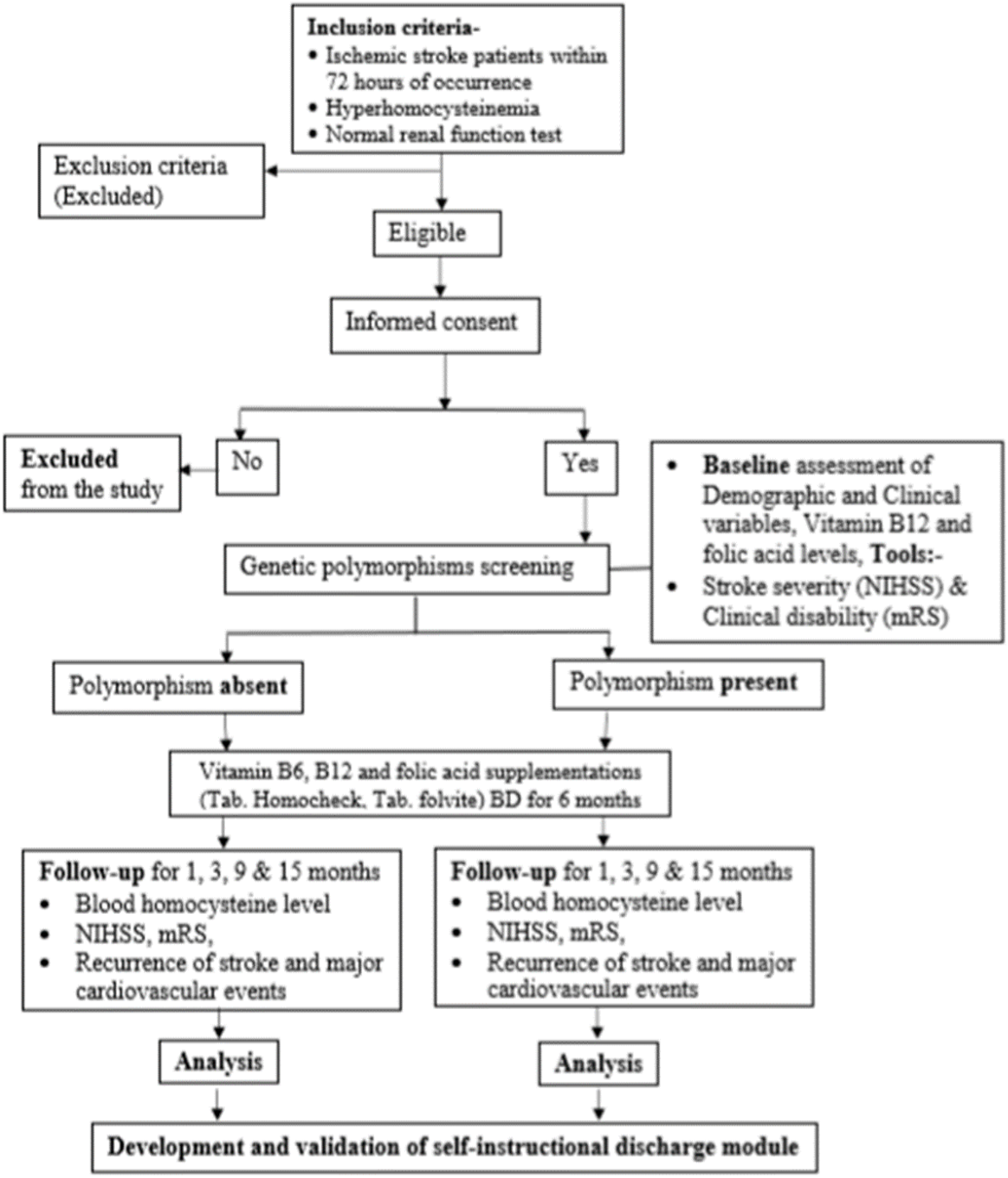
Flow chart


Inclusion criteria-
• Ischemic stroke patients within 72 hours of occurrence
• Hyperhomocysteinemia
• Normal renal function test
Exclusion criteria (Excluded)
Eligible
Informed consent
Excluded from the study
No
Yes
• Baseline assessment of Demographic and Clinical variables, Vitamin B12 and folic acid levels, Tools:-
• Stroke severity (NIHSS) & Clinical disability (mRS)
Genetic polymorphisms screening
Polymorphism absent
Polymorphism present
Vitamin B6, B12 and folic acid supplementations (Tab. Homocheck, Tab. folvite) BD for 6 months
Follow-up for 1, 3, 9 & 15 months
• Blood homocysteine level
• NIHSS, mRS,
• Recurrence of stroke and major cardiovascular events
Follow-up for 1, 3, 9 & 15 months
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Analysis
Analysis
Development and validation of self-instructional discharge module