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Prevalence, Clinical Profile and Outcome of Patients Presenting with Stroke

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

BACKGROUND: Stroke is a clinical condition that has a greatly increasing prevalence in the recent years owing to our 'modern' sedentary lifestyle. The aim of this study is to assess the prevalence, risk factors, clinical presentation and outcome of patients presenting with stroke in a tertiary care hospital.

METHODOLOGY: A prospective observational study was conducted in the Department of General Medicine, Saveetha Medical College & Hospital, Thandalam, Chennai for a period of 4 months, i.e., from January 2021 to April 2021.Stroke patients who met the eligibility criteria were included in the study. All sociodemographic and clinical data were recorded by reviewing the patient and his medical records regularly. Scores like NIHSS (National Institute of Health Stroke Scale) score and mRS (modified Rankin scale) scores were calculated and TOAST categorisation was done. Statistical analysis was done using Statistical Package for Social Sciences (SPSS) software. **RESULTS:** Out of 100 stroke patients, 65% were males and 35% were females. Mean age was 57.95 years. Ischemic strokes were seen in 85% of patients and the rest 15% had hemorrhagic stroke. The most common presentation was facial palsy (67%), followed by hemiparesis (62%) and

hemiplegia (38%). The important risk factors were hypertension (70%), diabetes mellitus (48%) and obesity (42%). Based on mRS scores, 59% of the patients had bad outcome. **CONCLUSION:** Hypertension and diabetes were the most common risk factor in our study population. Preventive measures include creating awareness, lifestyle changes and compliance to medications. National programmes on stroke prevention initiate and coordinate awareness campaigns to reduce the incidence, morbidity and mortality of stroke. Finally, a multidimensional approach involving the patient community, general population, medical fraternity and government authorities is essential to curtail this modern epidemic.

Keywords: Stroke; clinical presentation; risk factor; hypertension; diabetes; outcome.

ABBREVIATIONS

NIHSS score mRS score SPSS ECG	 National Institute of Health Stroke Scale score; Modified Rankin scale score; software Statistical Package for Social Sciences software; Electrocardiogram;
TTE	: Transthoracic Echocardiogram;
MRA	: Magnetic Resonance Angiography;
CTA	: Computed Tomography Angiography;
TIA	: Transient Ischemic Attack;
HRT	: Hormone Replacement Therapy;
IRB	: Institutional Review Board;
CT scan	: Computed Tomography scan;
MRI	: Magnetic Resonance Imaging;
HDL	: High Density Lipoprotein;
LDL	: Low Density Lipoprotein;
VLDL	: Very Low Density Lipoprotein;
AF	: Atrial Fibrillation;
TOAST category	: Trial of Org 10172 in Acute Stroke Treatment.

1. INTRODUCTION

Stroke is a clinical condition that has a greatly increasing prevalence in the recent years owing to our 'modern' sedentary lifestyle. The World Health Organisation defines stroke as a clinical syndrome consisting of rapidly developing clinical signs of focal (or global as in case of coma) disturbance of cerebral function lasting more than 24 hours or leading to death with no apparent cause other than vascular origin [1]. This definition includes ischemic strokes, intracerebral hemorrhages, subarachnoid hemorrhages and cerebral venous sinus thrombosis [2].Globally, about 68% of stroke cases are of ischemic type and 32% are of hemorrhagic type [3]. Stroke is the second leading cause of death globally, next to cardiovascular diseases and is a leading cause of adult disability [4,5]. The global burden of stroke is on the rise in terms of morbidity, mortality and disability.

Ischemic strokes occur when more than 80% of the cerebral vessel is occluded (most commonly

by a thrombosis) whereas hemorrhagic strokes occur following rupture of a cerebral vessel. Patients with hemorrhadic stroke usually present with acute onset headache, vomiting and high blood pressure along with focal neurological deficits that occur within minutes. Ischemic strokes progress gradually over hours and patients can present with paralysis, paresis, ataxia, vomiting and eye gaze. The clinical manifestations depend on the site of lesion. CT scan of the brain remains the first line imaging modality in order to distinguish between hemorrhagic and ischemic stroke. CT scan should be done immediately after admission as it plays a key role in guiding further management of the patient. Work up for stroke includes routine investigations followed by electrocardiogram (ECG), transthoracic echocardiogram (TTE), angiography (magnetic resonance angiography "MRA", computed tomography angiography "CTA", Carotid Doppler, 4-vessel angiography). Approximately 54% of strokes worldwide can be attributed to hypertension [6]. Table 1 shows the risk factors contributing to the development of stroke.

 Table 1. The risk factors contributing to the development of stroke

NON – MODIFIABLE RISK FACTORS	MODIFIABLE RISK FACTORS		
 Age Sex Race, ethnicity Family history of stroke or transient ischemic attacks (TIAs) 	 Hypertension Diabetes mellitus Hyperlipidemia Alcohol and drug abuse Smoking Physical inactivity Cardiac disease (Atrial fibrillation) OCP use/ Hormone replacement therapy (HRT) 		

Given the rising prevalence of stroke and limited access of majority of the Indian population to quality stroke care services, prevention strategies play a crucial role in reducing the burden of stroke in terms of morbidity, mortality and disability. But limitations to such strategies include lack of community awareness and also that most patients have multiple risk factors attributing to the development of stroke.

The aim of this study is to assess the prevalence, risk factors, clinical presentation and outcome of patients presenting with stroke in a tertiary care hospital.

2. MATERIALS AND METHODS

A prospective observational study was conducted in the Department of General Medicine, Saveetha Medical College & Hospital, Thandalam, Chennai for a period of 4 months, i.e., from January 2021 to April 2021.

2.1 Eligibilty Criteria

- Patients who had confirmed diagnosis of stroke (confirmed either by computed tomography scan 'CT SCAN' or magnetic resonance imaging 'MRI' of the brain)
- Patients admitted during the study period from January 1, 2021 to April 30, 2021.
- Patients who had complete medical records
- Patients from whom informed consent was obtained

Individuals who did not meet the eligibility criteria were excluded from the study.

Patients presenting with neurological deficits were identified as having stroke and managed for the same based on the guidelines given by the Ministry of Health and Family Welfare as a part of the National Programme for Prevention & Control of Cancer, Diabetes, Cardiovascular diseases & Stroke (NPCDCS).

All sociodemographic and clinical data including investigations performed, treatment regimens prescribed and outcomes were recorded by reviewing the patient and his medical records regularly during his stay in the hospital.

2.2 Physical Examination

The severity of stroke was assessed using National Institute of Health Stroke Scale (NIHSS) within 24 hours of admission of the patient and was classified as follows: score 0 – no stroke; score 1 to 4 – minor stroke; score 5 to 15 – moderate stroke; score 15 to 20 – moderate to severe stroke; score 21 to 42 – severe stroke. Vitals (blood pressure, temperature) were monitored and documented as well.

2.3 Laboratory and Radiological Investigations

Investigations that were performed include fasting and postprandial blood glucose levels, HbA1C, complete blood count, fasting lipid profile (total cholesterol, triglycerides, HDL, LDL, VLDL and cholesterol/HDL ratio), serum uric acid, electrolytes, blood urea, serum creatinine, electrocardiogram (ECG), echocardiogram. Imaging modalities used and findings on brain imaging and angiography were also documented.

2.4 Operational definitions

Hypertension: If the patient had history of hypertension or treatment with antihypertensive medications or blood pressure >140/90 mmHg 24 hours after onset of stroke.

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Diabetes mellitus: If patient was previously on oral hypoglycaemic agents/ insulin treatment or had the diagnosis of any type of diabetes or fasting blood sugar levels \geq 126 mg/dl or had a documented random blood sugar \geq 200 mg/dl or glycosylated hemoglobin of \geq 6.5%.

Dyslipidemia or hyperlipidemia: If patient had history of hyperlipidemia or using lipid lowering medication or total cholesterol ≥200 mg/dl, LDL cholesterol ≥100 mg/dl, HDL cholesterol <40 mg/dl for men or <50 mg/dl for women or serum triglyceride 150 mg/dl.

Obesity: According to WHO, Body Mass Index (BMI) >30 kg/m². Central obesity: Waist circumference more than 102cm in men and 88cm in women.

Smoker: On average, 2 cigarettes per day in men and 1 per day in women. Former smoker: who abstained from smoking for greater than 1 year. Current smoker: smoking within 1 year ago

Alcohol consumption: On average ≥ 2 drinks/day for males and ≥ 1 drinks/day for females (previous drinker: for more than 1 year).

2.5 TOAST Category

Large artery atherosclerosis: These patients have clinical and brain imaging findings of either stenosis (>50%) or occlusion of a major brain artery or branch cortical artery due to atherosclerosis.

Cardioembolism: This category includes patients with arterial occlusions due to embolisms arising from the heart in the absence of ipsilateral large vessel disease.

Small vessel occlusion (lacunar stroke): This category includes non-cortical strokes smaller than 16mm without a cardiac source of embolism or significant large vessel stenosis.

Other determined etiology: This category includes patients with rare causes of stroke such as nonatherosclerotic vasculopathies, hypercoagulable states or hematologic disorders.

Undetermined etiology: Cases with two or more possible etiologies or a negative/incomplete workup were included under this category.

2.6 Assessment of Outcome

The scale is scored from 0 to 6 where 0 indicates no symptoms and 6 indicates death. Good outcomes were defined as mRS scores 0-2 whereas poor outcomes were defined as mRS scores 3-6. All patients were reassessed 1 month after stroke onset to determine the outcome. Patients who did not turnup to the hospital were contacted over the telephone to assess the functional status/mortality.

This study focusses primarily on the prevalence, risk factors, clinical presentation and outcome of stroke patients. Though a detailed analysis of each type of stroke cannot be made owing to time constraints and inadequacy of data, maximum possible efforts have been made to analyse certain aspects of the study like clinical presentation and risk factor analysis based on the type of stroke.

2.7 Statistical Analysis

The presentation of the categorical variables was done in the form of number and percentage (%). On the other hand, the quantitative data were presented as the means \pm SD. The following statistical tests were applied for the results:

- 1. The comparison of the variables which were quantitative in nature were analysed using ANOVA and post hoc comparison was done using Bonferroni correction.
- 2. The comparison of the variables which were qualitative in nature were analysed using Chi-Square test. If any cell had an expected value of less than 5 then Fisher's exact test was used.

The data entry was done in the Microsoft EXCEL spreadsheet and the final analysis was done with the use of Statistical Package for Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, ver 21.0.

For statistical significance, p value of less than 0.05 was considered statistically significant.

3. RESULTS

A total of 100 stroke patients were included in the study. Among them, 65% were males and 35% were females. In the present study, 31% of patients belonged to age group 61-70 years followed by 51-60 years (27%) and only 12% of the patients belonged to the age group 31-40 years. Table 3 shows the distribution of socio-

demographic characteristics among the study subjects.

Table 2. The functional outcome in stroke patients was measured at discharge using modifiedRankin scale 'mRS'

Modified Rankin Scale (mRS)	Description
0	No symptoms
1	No significant disability. Able to carry out all usual activities despite some symptoms
2	Slight disability. Able to look after own affairs without assistance, but unable to carry out all previous activities
3	Moderate disability. Requires some help, but able to walk unassisted
4	Moderately severe disability. Unable to attend to own body needs without assistance, Unable to walk unassisted
5	Severe disability. Requires constant nursing care and attention, bedridden, incontinent
6	Dead

Table 3. Distribution of socio-demographic characteristics of study subjects

Socio-demographic characteristics	Frequency	Percentage	
Age(years)			
31-40	12	12.00%	
41-50	16	16.00%	
51-60	27	27.00%	
61-70	31	31.00%	
>70	14	14.00%	
Mean ± SD	57.95 ± 12		
Median(25th-75th percentile)	58.5(49-68)		
Range	32-82		
Gender			
Female	35	35.00%	
Male	65	65.00%	

Majority of patients (73%) suffered from thrombotic stroke followed by hemorrhagic stroke (15%). Embolic stroke occurred in 12% of

patients. Fig. 1 shows the distribution of type of stroke among study subjects.

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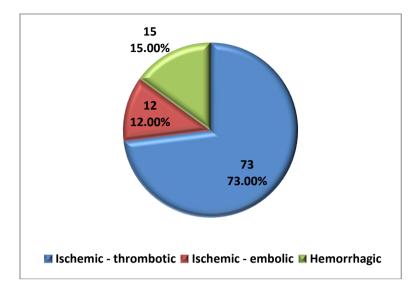


Fig. 1. Distribution of type of stroke among study subjects

In present study, the most common presentation among stroke patients was facial palsy (67%), followed by hemiparesis (62%) and hemiplegia (38%). Presentations like headache, dysphasia, altered consciousness and vomiting though present, were relatively less common. Fig. 2 and Table 2 show the distribution of presentation among study subjects.

On overall analysis, hypertension was the most common risk factor contributing to the development of stroke in 70% of patients followed by diabetes (48%) and obesity (42%). Hypertension was a significant risk factor in thrombotic (78.08%) and hemorrhagic stroke (86.67%). Embolic stroke was most commonly associated with valvular heart disease (91.67%) and atrial fibrillation (91.67%). Table 5 shows the distribution of risk factors among study subjects.

On analysing the NIHSS score at the time of admission, 73% of patients presented with moderate stroke and 17% with severe stroke. Table 4 and Fig. 3 show the distribution of NIHSS score among study subjects.

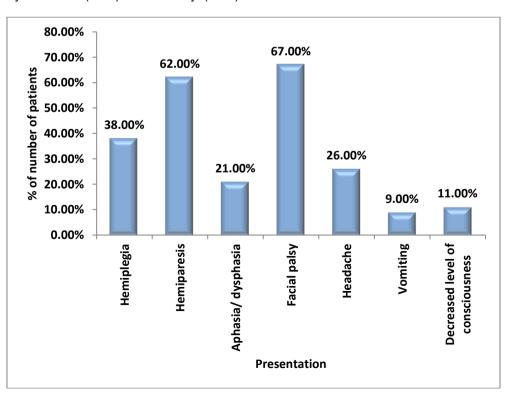


Fig. 2. Distribution of presentation of study subjects

Table shows the analysis of clinical presentation in individual stroke types.

 Table 4. Comparison of presentation between ischemic - thrombotic, embolic and hemorrhagic stroke

Presentation	Ischemic - thrombotic(n=73)	lschemic - embolic(n=12)	Hemorrhagic(n=15)
Hemiplegia	21 (28.77%)	10 (83.33%)	7 (46.67%)
Hemiparesis	52 (71.23%)	2 (16.67%)	8 (53.33%)
Aphasia/ dysphasia	13 (17.81%)	5 (41.67%)	3 (20%)
Facial palsy	58 (79.45%)	4 (33.33%)	5 (33.33%)
Headache	10 (13.70%)	2 (16.67%)	14 (93.33%)
Vomiting	0 (Ô%)	0 (0%)	9 (60%)
Decreased level of consciousness	0 (0%)	9 (75%)	2 (13.33%)

Table 5. Distribution of risk factors among study subjects

Risk Factors	Total	Ischemic - thrombotic(n=73)	Ischemic - embolic(n=12)	Hemorrhagic(n=15)	P value
Hypertension	70 (70%)	57 (78.08%)	5 (41.67%)	13 (86.67%)	0.005 [†]
Diabetes	48(48%)	37 (50.68%)	6 (50%)	5 (33.33%)	0.467 [†]
Hyperlipidemia	38(38%)	28 (38.36%)	5 (41.67%)	5 (33.33%)	0.894 [*]
Smoking	22(22%)	11 (15.07%)	6 (50%)	5 (33.33%)	0.012 [*]
Alcohol intake	29(29%)	18 (24.66%)	6 (50%)	5 (33.33%)	0.152 [*]
Obesity	42(42%)	30 (41.10%)	4 (33.33%)	8 (53.33%)	0.553 [†]
Family history	17(17%)	12 (16.44%)	2 (16.67%)	3 (20%)	0.91 [*]
of stroke or	. ,	. ,	. ,		
TIAs					
Ischemic heart	3(3%)	3 (4.11%)	0 (0%)	0 (0%)	1
disease		. ,	. ,	. ,	No p
					value
Atrial	11(11%)	0 (0%)	11 (91.67%)	0 (0%)	<.0001
fibrillation					H:No p
					value
Valvular heart	14(14%)	3 (4.11%)	11 (91.67%)	0 (0%)	<.0001
disease	. ,	. ,	. ,		
Heart failure	5(5%)	5 (6.85%)	0 (0%)	0 (0%)	0.783 [*]
		. ,		• •	H:No p
					value
OCP/ HRT use	7(7%)	6 (8.22%)	1 (8.33%)	0 (0%)	0.672 [*]

Fisher's exact test, ^{\dagger} Chi square test

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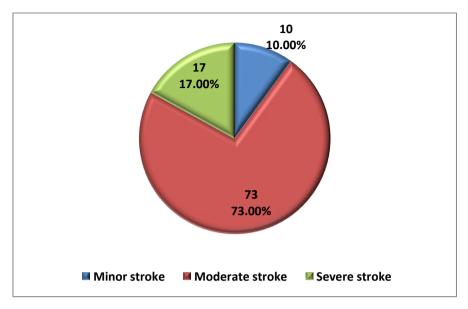


Fig. 3. Distribution of NIHSS score of study subjects

NIHSS score	Frequence	cy Percentage
Minor stroke	10	10.00%
Moderate stroke	73	73.00%
Severe stroke	17	17.00%
Mean ± SD	13.16 ± 9	.06
Median(25th-75th percentile)	11(8-13)	
Range	3-40	
In present study, family history of stroke, present in 18 out of 100 patients (18%). In majority (78.82%) of the patients, category for ischemic stroke was larg atherosclerosis followed by cardioe (14.12%). Table 6 shows the distrib TOAST category for ischemic Table 7 shows the distribution of category for ischemic stroke among th subjects.	TOAST e artery mbolism ution of stroke. TOAST	In present study, majority (59%) of patients had bad outcome and 41 out of 100 patients (41%) had good outcome. Mean value of mRS score of study subjects was 2.79 ± 1.15 with median (25th-75th percentile) of 3(2-3). It is shown in Fig. 4. The outcome of ischemic stroke patients was correlated with the TOAST category they belonged to and the results are shown in Table 8.

TOAST category for ischemic stroke	Frequency	Percentage
Cardioembolism	12	14.12%
Large artery atherosclerosis	67	78.82%
Other determined etiology	5	5.88%
Small vessel occlusion	1	1.18%
Total	85	100.00%

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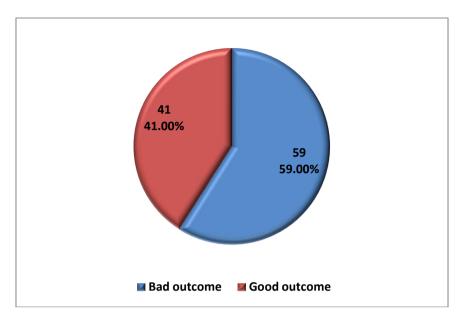


Fig. 4. Distribution of outcome (mRS score) of study subjects

Outcome (mRS score)	Cardioembolis m(n=12)	Large artery atherosclerosis(n =67)	Other determined etiology(n= 5)	Small vessel occlusio n(n=1)	Total	P value
Bad	5	45	2	0	52	0.091
outcome	(41.67%)	(67.16%)	(40%)	(0%)	(61.18%)	
Good	7	22	3	1	33	
outcome	(58.33%)	(32.84%)	(60%)	(100%)	(38.82%)	
Mean ± SD	2.83 ± 1.59	2.81 ± 1.03	2.2 ± 0.84	2 ± 0	2.76 ± 1.11	0.599 [‡]

4. DISCUSSION

In our study, ischemic stroke (thrombotic type) (73%) was the most prevalent type followed by hemorrhagic stroke (15%). This indicates that patients with ischemic stroke would have had significant exposure to modifiable risk factors and knowledge regarding controlling the same through lifestyle modifications could have prevented the current condition. These findings coincide with the study done by Temesgen et al who also reported that ischemic stroke (65.8%) was the most frequently diagnosed type followed by hemorrhagic stroke (21.9%) [1].

The mean age of stroke patients was 57.95years in our study. This finding almost correlates with the mean age value of 56.25 years reported by Nandigam et al. [7]. Dayapoglu et al reported a much higher mean age of 64.18 ± 7.91 years in his study [8]. Increasing age, being a common risk factor for many chronic diseases like cancer,

Fisher's exact test, ‡ ANOVA
cardiovascular diseases and neurodegenerative
diseases, attributes to increased risk of stroke as
well. A study done by Roger et al states that the
incidence of stroke doubles for each decade after
the age of 55 [9]. This can be explained by the
increased prevalence other risk factors such as
diabetes mellitus (type 2), hypertension and
atherosclerosis with advancing age [1].

Among our study group of stroke patients, 65% were males and 35% were females. Kamal et al has also reported a similar observation in his study i.e., 76% males and 24% females with male to female ratio of 3:1 [10]. Suchgender differences can be attributed to lifestyle activities like smoking, alcohol consumption and also greater prevalence of myocardial infarction and arterial disorders among males [11].

In our study, the common clinical presentation in thrombotic stroke was facial palsy (79.4%) and hemiparesis (71.2%) whereas hemorrhagic

stroke patients commonly presented with headache (93.3%). vomitina (60%) and hemiparesis (53.3%). Aphasia was present in 21% of stroke patients and the values were not statistically significant. Fekadu et al had reported headache (71.7%), aphasia (60%) and facial palsy (58.3%) as the common presentation in ischemic stroke and headache (78.6%), aphasia (60.7%) and vomiting (57.1%) as the common presentation in hemorrhagic stroke in their study [12].

In our study, hypertension was the most common risk factor present in 70% of stroke patients. Similarly, most of the stroke studies report hypertension as the leading risk factor as observed by Abdullah et al in 86.6% [13] and Al-Hashel et al. in 80.9% of stroke patients [14] in their respective studies. In hypertensives, vascular remodelling, inflammation, oxidative stress and baroreflex dysfunction play major roles towards development of stroke. On analysing the frequency of hypertension in various types of stroke, it was present in 72.94% of ischemic stroke patients and 86.67% of hemorrhagic stroke patients in our study. Comparing this with the observations made by Tirschwell et al., hypertension was present in 94.5% of ischemic stroke patients and 98% of hemorrhagic stroke patients in their study [15]. Such rising trends could be due to lack of awareness among patients, lack of active screening programs, non-compliance to drug therapy and poor follow-up by patients. The SimCard study done by Tian M et al; proves the importance of such awareness and intervention programmes. Participants of the SimCard study were managed by community health workers through an Android-powered app on a monthly basis focusing on use of 2 medications (blood pressure-lowering agents and aspirin) and 2 lifestyle modifications (smoking cessation and salt reduction). The results indicated that the simplified cardiovascular management program improved the quality of primary care and clinical outcomes in the intervention group [16]. Collins et al. had reported in his study that a 5-6mmHg reduction in the blood pressure lowered the relative risk of stroke by 42% [17]. Hypertension is the most powerful modifiable factor and the second most powerful risk factor, after age, for stroke, regardless of geographic location and ethnicity [18].

In our study, 48% of stroke patients were diabetic. This finding almost correlates with the study done by Habibi-Koolaee et al which reports

36% of diabetics among stroke patients [19] whereas Abullah et al reported a much higher frequency of diabetes (62.2%) among stroke patients [13]. Onanalysing the same in individual stroke types, the prevalence of diabetes was higher in ischemic stroke (50.6%) compared to hemorrhagic stroke (33.3%). Lau et al reported a similar higher prevalence in ischemic stroke (33%) compared to hemorrhagic stroke (26%) [20]. Diabetes leads to stroke by causing vascular endothelial dysfunction, increased early-age arterial stiffness, systemic inflammation and capillary basal membrane thickening [21].

Cigarette smoking is a well-known risk factor for stroke which has a dose-response relationship between number of cigarettes smoked and the risk of developing stroke. Smoking was present in 22% of stroke patients in our study. This is much higher compared to the study done by Gedefa B et al which states smoking as a risk factor in only 8% of stroke patients [22].

In our study 17% of stroke patients had family history of stroke/ TIAs. This is much higher than the value (4.4%) observed by Alemayehu et al. [23]. Atrial fibrillation is a disorder of heart rhythm that affects 33 million people worldwide [24]. Incident stroke related to atrial fibrillation (AF) has nearly tripled in the past three decades signifying that AF is a major contributor to embolic stroke [25]. In our study, AF contributed to the development of embolic stroke in 91.6% of patients. In our study, AF was present 11% of stroke patients. This value almost correlates with the study done by Gedefa B et al., in which the incidence of AF was 14.7% [22].

On analysing the TOAST category, a major proportion of ischemic strokes was attributed to large artery atherosclerosis (78.82%) in our study. Whereas in the ARCOS – IV (Auckland Regional Community Stroke Study) study done by Krishnamurthi RV et al, almost all categories had a fair share of cases with maximum being undetermined etiology (31%) followed by cardioembolism (29%) [26]. This trend could be attributed to the cultural, ethnic and racial differences in the population as well as the accessibility to health care services and advancements in the medical field in their country.

On analysing the outcome of stroke patients using mRS score, 41% of patients had good outcome whereas 59% of patients had bad outcome in our study. In a study done by Qawasmeh et al. frequency of good and bad outcomes was equal in distribution [27]. The outcome among patients recovering from stroke can be variable. This can be explained by the fact that the outcome is influenced by various factors like age, presence of comorbidities, type of stroke, time of admission to hospital after stroke onset, severity of stroke and Glasgow coma scale (GCS) at the time of admission, whether appropriate treatment was instituted, patients' response to treatment and compliance to drugs and physiotherapy post-treatment. The limitation of this study was that the study population comprised of stroke patients living in southern India. Therefore, generalization of these findings to individuals living in other geographic regions is limited.

5. CONCLUSION

Many of the risk factors for stroke are modifiable and preventive measures include awareness among patients as well as general population, lifestyle changes, compliance to medications and regular follow up. National programmes on stroke prevention like irb have been developed to coordinate public initiate and awareness campaigns and develop more guidelines to reduce the incidence, morbidity and mortality of stroke in India. Thus, a multidimensional approach involving the patient community, general population, medical fraternity and government authorities is essential to curtail this modern epidemic. However, primordial prevention which aims at preventing the emergence of risk factors in a population by individual and mass education seems to be the most effective strategy. But owing to the vast population in our country and that a significant fraction of it is residing in rural areas, this is difficult to achieve. Hence, primary prevention involves intervention in the prewhich pathogenesis phase before the disease onset, by identifying the risk factors and employing specific protective measures is the most effective prevention strategy.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethical approval for the study was granted by the Institutional Review Board (IRB).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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