Sir,

Stroke can be both arterial and venous and etiologically diverse. Rising incidence of stroke in young adults and post-stroke hospitalization pose a severe burden to the family, society, and hospitals.[1] In addition to established risk factors (obesity, metabolic disorders, atherosclerosis, etc.), gut microbiota critically influence pathogenesis of stroke.[2] While gut microbiota affect acute cerebral ischemia via bidirectional microbiota-gut-brain axis,[2] its role in enhancing arterial thrombosis has been studied but not adequately in intracranial sino-venous thrombosis (IST). Venous thrombosis is driven by an imbalance between prothrombotic and thrombolytic processes.[3] We hypothesize that the gut microbiota in IST and acute ischemic stroke in the young (15 to 49 years) (AIS) differs from that of healthy volunteers (HV). This cross-sectional observational study (January 2016 to January 2018) at a tertiary care hospital in South India, compares the composition and diversity of gut microbiota between patients with IST, AIS, and HV (Institutional ethics committee approval: IEC 270/2015).

Inclusion criteria for IST: 1) Confirmed diagnosis with radiological imaging. 2) Recruited within one month of the onset of symptoms. 3) Patients >18 years of either gender. 4) Willingness to participate. Inclusion criteria for AIS were: 1) Infective aetiology. 2) Antibiotics usage until processed further. The time difference between the onset of stroke and collection of stool samples was 6 (6.5) days (Median ± IQR). The stool samples were thawed, homogenized, and DNA was extracted using Qiagen DNA stool mini kit. Integrity and quality of DNA were checked using nanodrop spectrophotometer (ThermoFisher Scientific, USA). The fecal gut samples were sequenced via nanopore sequencing platform for 16S rRNA amplicon sequencing.

The study recruited 14 patients each in case groups (IST and AIS) and HV. Baseline demographics are in Supplementary Table 1.

Differences in abundance of phyla between groups were maximum for Actinobacteria (p = 0.031), Chlorobi (p = 0.011), Proteobacteria (p = 0.022), and Tenericutes (p = 0.009). Proteobacteria was significantly higher in IST whereas Actinobacteria, Cholorobi, and Tenericutes were lower in IST and AIS than HV. At Genus level, IST had greater abundance of *Acetobacterium, Citrobacter, Enterobacter, Erwinia, Pantoaea, Xenorhabdus*, etc. HV had greater abundance of *Aerococcus, Citrobacter, Enterobacter, Clostridium, Eubacterium, Pseudobutyrivibrio, Robinsoniella, Roseburia, Ruminococcus*, etc. [Figure 1a and b]. Firmicutes: Bacteroidetes ratio (F:B) was higher in IST (7.01) than AIS (3.64) and HV (3.94).

Total read counts of 613836 were clustered to 900 OTUs. The α-diversity, reflecting bacterial community richness, and evenness (Chao1 and Shannon index), were similar in IST, AIS, and HV. The β-diversity, (similarity of the gut microbiota structures) evaluated by principal coordinates analysis (PCoA) derived from the Bray–Curtis distances, indicated that microbiota structure differed significantly between the IST, AIS, and HV (p < 0.013) [Figure 2].

To the best of our knowledge, this is the first report from India comparing the composition and diversity of the gut microbiota in IST and AIS, highlighting the following differences: 1) IST harboured more opportunistic pathogens in family Enterobacteriaceae and Phylum Proteobacteria than AIS and HV. 2) The F: B ratio was also higher in IST and 3) Short-chain fatty acids (SCFAs) producing genus *Butyrivibrio, Roseburia, Ruminococcus, Eubacterium, Pseudobutyrivibrio* were significantly lower in IST and AIS than in HV.

In our study, IST group had more opportunistic pathogens belonging to family Enterobacteriaceae, could be a new
Figure 1: Comparison of the gut microbiota pattern between persons with intracranial sino-venous thrombosis (IST), acute ischemic stroke in the young (AIS), and the healthy volunteers (HV): (a) Phylum level (b) Genus level
biodarker in predicting clinical outcome in AIS (all age groups).[6] IST also exhibited higher F:B ratio than AIS and HV. The F:B ratio is a sign of gut dysbiosis. The ratio of F:B could help prevent the occurrence of stroke and its complications.[3] However, further research into the role of F:B ratio is required to unravel whether a high or low ratio is beneficial.

Mohammed et al.[3] showed that gut microbiota affects coagulation in humans through a single healthy donor Fecal Microbiota Transplantation (FMT) in pathological hypercoagulable states. FMT modestly suppressed the onset of thrombin generation in metabolic syndrome. Although vitamin K-producing bacteria would potentially raise vitamin K levels, it is unlikely to precipitate venous thrombosis.[6] We found that vitamin K-producing Citrobacter (P = 0.02) and Enterobacter (P = 0.02), though significantly more abundant in IST, may not have contributed.

The α-diversity (ChaO1 and Shannon index) between the groups in our study did not show any significant differences, similar to three previous clinical studies comparing microbiomes in cerebral ischemia and HV.[7-9] However, Wang et al.[10] showed lower and Yin et al.[11] showed significantly higher α-diversity in cases than HV. The β-diversity was significantly different between the groups (p < 0.013), similar to observations in other studies,[10,11] but contradicted by two others.[8,9]

Cerebral ischemic stroke is associated with a decline in SCFAs (acetate, propionate, and butyrate) produced by anaerobic fermentation of dietary fibres.[12] Moreover, transplanting SCFA-producing fecal microbiota was useful in cerebral ischemic stroke.[13] In our study, IST and AIS had significantly fewer SCFA-producing microbiota (Butyribrio, Roseburia, Ruminococcus, Eubacterium, and Pseudobutyribrio; under Phylum Firmicutes) than HV. Although a Chinese study contradicted this,[9] Li et al.[9] demonstrated reduction in butyrate-producing bacteria in stroke. Limitations of our study include small sample size within a single centre, dietary heterogeneity, socioeconomic diversity, and inability to confirm causal relationships between gut microbiota dysbiosis and IST and AIS.

In summary, our findings suggest that SCFA-producing bacteria diminish in both IST and AIS than HV, highlighting the microbiota gut-brain axis, and need for more microbiome research in venous stroke. Our findings also helps generate hypotheses for future investigations, offers clues on sample size for larger studies, and proposes FMT for remodelling the microbiota for ameliorating the brain ischemic response.

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Conflicts of interest
There are no conflicts of interest.

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### Supplementary Table 1: Demographics of the study population, stroke severity, and outcome

|                      | IST ($n=14$) | AIS ($n=14$) | HV ($n=14$) | $P$  |
|----------------------|--------------|--------------|-------------|------|
| **Age**              |              |              |             |      |
| Range, years         | 20–47        | 20–48        | 22–45       |      |
| Mean±SD, years       | 28.86±8.84   | 34.79±8.37   | 31.57±6.96  | 0.167|
| **Gender**           |              |              |             |      |
| Male, $n$ (%)        | 11 (78.6)    | 13 (92.9)    | 10 (71.4)   |      |
| Female, $n$ (%)      | 3 (21.4)     | 1 (7.1)      | 4 (28.6)    | 0.483|
| **Socioeconomic status*, $n$ (%)** |            |              |             |      |
| Lower-middle class   | 9 (64.3)     | 9 (64.3)     | 6 (42.9)    |      |
| Upper-lower class    | 3 (21.4)     | 3 (21.4)     | 1 (7.1)     |      |
| Upper-middle class   | 2 (14.3)     | 2 (14.3)     | 7 (50)      | 0.206|
| **Dietary habits, $n$ (%)** |          |              |             |      |
| Vegetarian           | 6 (42.9)     | 4 (28.6)     | 4 (28.6)    |      |
| Mixed diet           | 8 (57.1)     | 10 (71.4)    | 10 (71.4)   | 0.772|
| **Stroke severity as per NIHSS, $n$ (%)** |           |              |             |      |
| No stroke symptoms (NIHSS: 0) | 10 (71.42) | 1 (7.14)     | NA          | 0.005|
| Minor stroke (NIHSS: 1-4) | 2 (14.28) | 6 (42.85)    |             |      |
| Moderate stroke (NIHSS: 5-20) | 2 (14.28) | 5 (35.71)    |             |      |
| Moderate/Severe stroke (NIHSS: 21-42) | 0 | 2 (14.28) |            |      |
| **Stroke outcome as per mRS** |          |              |             |      |
| Good outcome (MRS: 0-2) | 7 (50)      | 4 (28.57)    | NA          | 0.246|
| Poor outcome (MRS: 3-5) | 7 (50)     | 10 (71.42)   |             |      |

IST=Intracranial sino-venous thrombosis, AIS=Acute ischemic stroke in young, HV=Healthy volunteers, NIHSS=National Institutes of Health Stroke Scale, mRS=Modified rankin scale. *Oberoi SS. Updating income ranges for Kuppuswamy’s socioeconomic status scale for the year 2014. Indian J Public Health 2015;59:156-7