PREDICT-EV; Are Astrocyte derived Extracellular Vesicles present in the circulation of Transient Ischaemic Attack patients?

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BACKGROUND

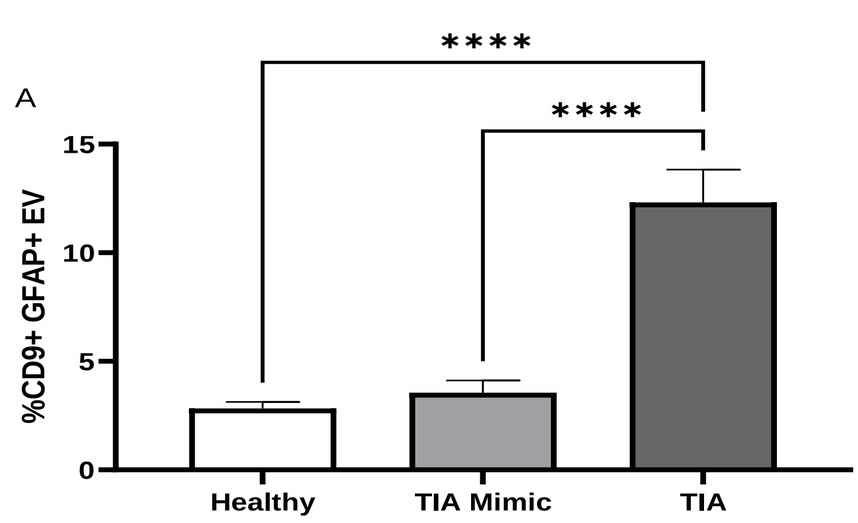
- Stroke is the second leading cause of death worldwide and is the fourth in the UK. An uncharacterised increase in risk of suffering a disabling stroke is associated with transient ischaemic attack (TIA) patients. This risk is highest in the 24 hours following a TIA, but persists at 7 days (8%), 1 month (11.5%), 3 months (17.3%) and 12 months (25%). A major hallmark of ischaemic stroke is blood-brain barrier (BBB) disruption.
- The neurovascular unit (NVU) that comprises the BBB is crucial to maintaining homeostasis of the brain microenvironment. Two of the most integral cell types in the NVU are endothelial cells and astrocytes. Extracellular vesicles (EVs) are sub-micron particles produced by all cell types as part of normal homeostasis, but under stress conditions have been linked with pathophysiological responses.
- Disruption to the BBB causes leakage and due to their size, it is plausible that neural EVs normally restricted to the abluminal side can transverse the barrier to the vessel lumen.

To determine if Astrocyte derived EVs are present in the circulation of Transient Ischaemic Attack Patients.

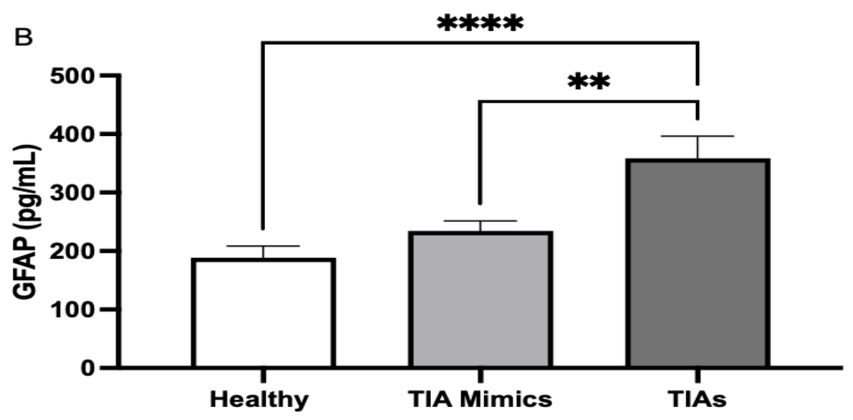
METHODS

- TIA patients were recruited from TIA clinic at Prince Charles Hospital Merthyr (N=150) and were compared to a 'TIA mimic' group (N=30) and a group of healthy controls (N=30).
- EVs were isolated from a-cellular plasma using size exclusion chromatography and characterised using Nanosight Tracking Analysis and flow cytometry. Confirmation of EV character was determined by CD9 antigen positivity and secondly stained for glial fibrillary acidic protein (GFAP) that represents astrocyte origin.
- An enzyme-linked immunosorbent assay (ELISA) was used to determine levels of free GFAP protein in plasma, that has been found to be increased following astrocyte reactivity

RESULTS



- The proportion of GFAP+ EV was significantly elevated in TIA patients compared to TIA mimics and healthy controls ($p<0.0001$).



- Plasma GFAP protein levels were significantly increased in TIA patients compared to both TIA mimics ($p<0.01$) and healthy controls ($p<0.0001$), reflective of astrogliosis.

CONCLUSIONS

Astrocyte derived EV are increased in the circulation of TIA patients. Whether the presence of GFAP+EV in circulation impacts stroke risk and if they have an impact on clotting processes is still under investigation.

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