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RESEARCH ARTICLE
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MICROBES AS A SOURCE OF NEURO-INFLAMMATION IN ALZHEIMER'S DISEASE: A PILOT ANALYSIS OF BACTERIAL LOAD, AB40, AND VIMENTIN IN TEMPORAL CORTEX AND CHOROID PLEXUS

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ABSTRACT

Background: Alzheimer's disease (AD) is increasingly linked to chronic neuro-inflammation, but the triggers that initiate and sustain this response remain elusive. Mounting evidence shows bacterial DNA and viable pathogens in AD brains, with vascular-adjacent structures such as the choroid plexus (CP) posited as portals of entry. We explored total bacterial burden, amyloid-β40 (Aβ40) concentration and vimentin dynamics in matched temporal cortex (TC) and CP from AD and control brains.

Methods: Frozen TC (Brodmann area 21) and CP samples from the South-West Dementia Brain Bank (6 controls, 7 AD) were processed under sterile conditions. Total DNA was extracted, quantified by β-globin qPCR, and assayed for bacterial 16S rRNA gene copies with contamination-minimised reagents. Aβ40 in guanidine-solubilised pellets was measured by sandwich ELISA; soluble vimentin and phospho-Ser56-vimentin were assessed by Western blot. Statistics included Welch's *t*-tests, two-way ANOVA and Pearson correlation.

Results: Mean log10 bacterial copies per 50 ng DNA were significantly higher in TC of AD cases (5.29 ± 0.53) than controls (4.49 ± 0.37; p=0.014); no difference emerged in CP. Two-way ANOVA showed a strong tissue effect (TC > CP, p=0.008) without group–tissue interaction. Aβ40 concentrations trended higher in AD TC (32 044 pg μL^{-1}) versus controls (10 424 pg μL^{-1}, p=0.098). Vimentin immunoblots revealed greater total and phospho-vimentin in AD, especially in CP. Bacterial load did not correlate with Aβ40 (r = 0.13, *NS*).

Conclusion: TC harbours a markedly elevated bacterial burden in AD, supporting the hypothesis that microbes contribute to regional neuro-inflammation. CP shows abundant vimentin but modest bacterial DNA, suggesting barrier activation rather than colonisation. Larger cohorts and species-specific assays are warranted to delineate causal links.

Keywords: Alzheimer's disease; choroid plexus; temporal cortex; bacteria; amyloid-β40; vimentin; neuro-inflammation.

INTRODUCTION

Persistent, sterile neuro-inflammation is now recognised as a defining feature of Alzheimer's disease (AD), yet the exogenous and endogenous stimuli that ignite and perpetuate this immune milieu remain contentious. Classical amyloid cascade and tauopathy frameworks posit that aberrant production, misfolding and spread of host proteins are sufficient to activate glia; however, these models neither explain the striking regional variability of pathology nor the frequent co-existence of systemic

inflammatory morbidities. Convergent microbiological evidence is re-energising an "infectious aetiology" hypothesis that was first floated nearly a century ago but eclipsed by protein-centric paradigms. Dominy *et al.* recently detected gingipain-positive *Porphyromonas gingivalis*—a keystone periodontopathogen—in >90 % of AD hippocampi and showed that its cysteine proteases cleave tau at neurotoxic sites and trigger neuronal death in murine models [1]. Parallel 16S-rRNA surveys in independent cohorts report significantly richer brain bacterial α-diversity and greater total microbial DNA in AD compared with age-matched controls [2], with oral taxa such as *Treponema* and gut-derived *Escherichia/Shigella* dominating the diseased metagenome. Mechanistic links between microbes and hallmark AD lesions are becoming increasingly plausible. Amyloid-β (Aβ) is now framed as an evolutionarily conserved antimicrobial peptide that oligomerises on microbial cell walls, thereby entrapping invaders at the cost of seeding extracellular plaques [3]. Likewise, tau hyper-phosphorylation can be induced by endotoxin-driven activation of p38 and JNK kinases, providing a conduit from peripheral infection to intracellular tangle formation. Cultivable *Chlamydia pneumoniae* has been isolated from AD frontal cortex, where bacterial inclusions co-localise with both neuritic plaques and neurofibrillary tangles [4]. Importantly, cytoskeletal vimentin—a type III intermediate filament—has emerged as a 'double-agent' in this context: diverse bacteria bind surface-exposed vimentin to gain cellular entry, while infection reciprocally drives vimentin phosphorylation, secretion and filament disassembly, processes that amplify inflammasome activation and microglial chemotaxis [5]. The anatomical gateways through which microbes or their molecular fragments breach central nervous system (CNS) privilege are under intense scrutiny. The blood–brain barrier (BBB) is often portrayed as the principal sentinel, yet the choroid plexus (CP), a highly vascularised stroma capped by a monolayer of epithelial cells forming the blood–cerebrospinal-fluid barrier (BCSFB), may represent an equally, if not more, permissive interface. Age- and AD-related remodelling of CP tight junctions, basement-membrane thickening and epithelial senescence collectively erode barrier integrity and foster leukocyte trafficking [6]. Vascular Aβ40—the predominant vascular isoform—accumulates early in CP, can trigger inflammation in endothelial cells by increasing the expression of inflammatory cytokines, promotes neurotoxic effects, and appears to have a role in microbial adhesion all of which have been implicated in AD pathology [7]. Recent studies indicate that periodontitis induces gut microbiota dysbiosis, which in turn dysregulates oral-gut-brain axis; the recolonisation with periodontal microbiota thus appears to accelerate plaque deposition specifically along the ventriculo-hippocampal axis, supporting a CP-centred route of dissemination [8]. Despite these insights, quantitative comparisons of bacterial burden across spatially distinct brain regions—and, crucially, their relationships to Aβ isoforms or host cytoskeletal responses—remain scarce. Resolving whether the CP merely filters circulating microbial fragments that subsequently lodge in downstream parenchyma, or whether it hosts occult colonisation that seeds propagative infection, carries therapeutic implications: the former scenario would prioritise systemic antimicrobial or immune-modulatory strategies, whereas the latter would motivate barrier-targeted interventions. Moreover, dissecting how vimentin dynamics integrate microbial sensing with cytoskeletal remodelling could unveil druggable nodes that decouple host defence from neuro-toxic propagation. To address these gaps, we undertook a pilot, region-matched analysis of total bacterial DNA, Aβ40 concentration and vimentin status (total and Ser56-phosphorylated) in temporal cortex (TC) and CP specimens from clinically and pathologically confirmed AD and control donors. We hypothesised that: (i) bacterial burden would be greater in AD than in controls; (ii) the CP, as a putative portal of entry, would harbour equal or higher bacterial loads relative to TC; and (iii) elevations in bacterial DNA would coincide with up-regulation and hyper-phosphorylation of vimentin, reflecting a coordinated host response to microbial challenge. By integrating microbial quantification with biochemical and immuno-histochemical read-outs, our study aims to refine the infectious hypothesis of AD and identify CP-centric biomarkers or checkpoints amenable to therapeutic modulation.

MATERIALS AND METHODS

Brain tissue: Frozen TC (BA21) and CP specimens from 7 AD and 6 control donors (ethical approval ITA058, South-West Dementia Brain Bank, UK) were age- and post-mortem-delay matched.

DNA extraction and quality control: Fifty–100 mg tissue aliquots were homogenised in phenol–chloroform under class II laminar flow. DNA pellets were ethanol-precipitated, resuspended in TE (pH 8.0) and quantified by QuantiFluor dsDNA assay. Purity (A260/280 ≥ 1.7) was verified by NanoPhotometer.

Quantification of total bacteria: β-globin qPCR normalised sample input (≈50 ng DNA per reaction). Total bacterial 16S copies were measured using Molzym 16S Basic MasterMix with primers 27F/338R and FAM-TAMRA probe; reactions ran on ABI StepOnePlus (95 °C 5 min; 40 cycles 95 °C 30 s, 60 °C 45 s). Standard curves employed *E. coli* genomic DNA (10^8–10^2 copies).

Aβ40 ELISA: Guanidine-solubilised pellets (100× dilution for TC; 500× for CP) were assayed with a human Aβ40 sandwich kit following manufacturer's instructions; absorbance was read at 450 nm with 540 nm correction.

Western Blotting: RIPA-soluble proteins (40 µg) were resolved on 12 % SDS-PAGE, transferred to nitrocellulose and probed with mouse anti-vimentin (1:5000) or rabbit anti-phospho-Ser56-vimentin (1:1 000), followed by HRP-IgG and ECL detection. β-Actin (42 kDa) served as loading control.

Statistics: Group comparisons used Welch's *t*-test; tissue and group effects were analysed by two-way ANOVA. Pearson correlations tested associations between bacterial load and Aβ40. $p < 0.05$ was considered significant (GraphPad Prism 10).

RESULTS

Total bacterial copies per 50 ng DNA in TC ranged from 10^4 to $>10^6$, with AD cases clustering at the upper end (Table 1). Welch's *t*-test confirmed a 0.8-log10 increase in AD (95 % CI 0.19–1.39, p=0.014). By contrast, CP bacterial loads were low (10^3–10^4) and overlapped between groups (Table 2). Two-way ANOVA highlighted a significant tissue main effect ($F_{1,22}$ = 8.74, p=0.008) without group–tissue interaction (Table 3), indicating that tissue type—not disease status—predominantly drives bacterial abundance. ELISA revealed a three-fold elevation of mean Aβ40 in AD TC (32 044 pg μL^{-1}) versus controls (10 424 pg μL^{-1}), albeit short of statistical significance (p=0.098). CP Aβ40 levels were quantifiable in only 5 subjects, showing no consistent group pattern. No correlation emerged between log10 bacterial load and Aβ40 in TC (r = 0.13, p=0.707; Table 4). Western blots consistently displayed four vimentin bands (~54–45 kDa) in TC and CP. Total vimentin intensity appeared greater in AD, especially in CP (Figure 1). Phospho-Ser56-vimentin was detectable in all CP samples and selectively in AD TC under high-stringency conditions, suggesting infection-responsive post-translational modification. Actin loading confirmed equal protein input, yet CP showed intrinsically lower actin signal than TC (Figure 2).

TABLE 1: TEMPORAL CORTEX—TOTAL BACTERIAL LOAD

Measure	Control	AD
n	6	6
Mean (log10 copies / 50 ng)	4.49	5.29
SD	0.37	0.53
95 % CI	4.10-4.88	4.73-5.85
Welch *t*	–	3.03
p-value	–	0.014

TABLE 2: CHOROID PLEXUS—TOTAL BACTERIAL LOAD

Measure	Control	AD
n	6	7
Mean (log10 copies / 50 ng)	2.85	3.56
SD	2.23	1.62
95 % CI	0.51-5.19	1.99-5.13
Welch *t*	–	0.65
p-value	–	0.54

TABLE 3: TWO-WAY ANOVA ON LOG10 BACTERIAL LOAD

Effect	F (df_1,df_2)	*p*-value
Group (AD vs Control)	1.72 (1, 22)	0.204
Tissue (TC vs CP)	8.74 (1, 22)	0.008
Interaction	0.01 (1, 22)	0.932

TABLE 4: CORRELATION IN TEMPORAL CORTEX (LOG10 BACTERIA × AB40)

Statistic	Value	*p*-value
n	12	–
Pearson *r*	0.13	0.707
95 % CI	–0.46 to 0.65	–

FIGURE 1: RELATIVE DENSITOMETRY OF TOTAL VIMENTIN BANDS IN TEMPORAL CORTEX (TC) AND CHOROID PLEXUS (CP), SHOWING INCREASED EXPRESSION IN AD, ESPECIALLY IN CP

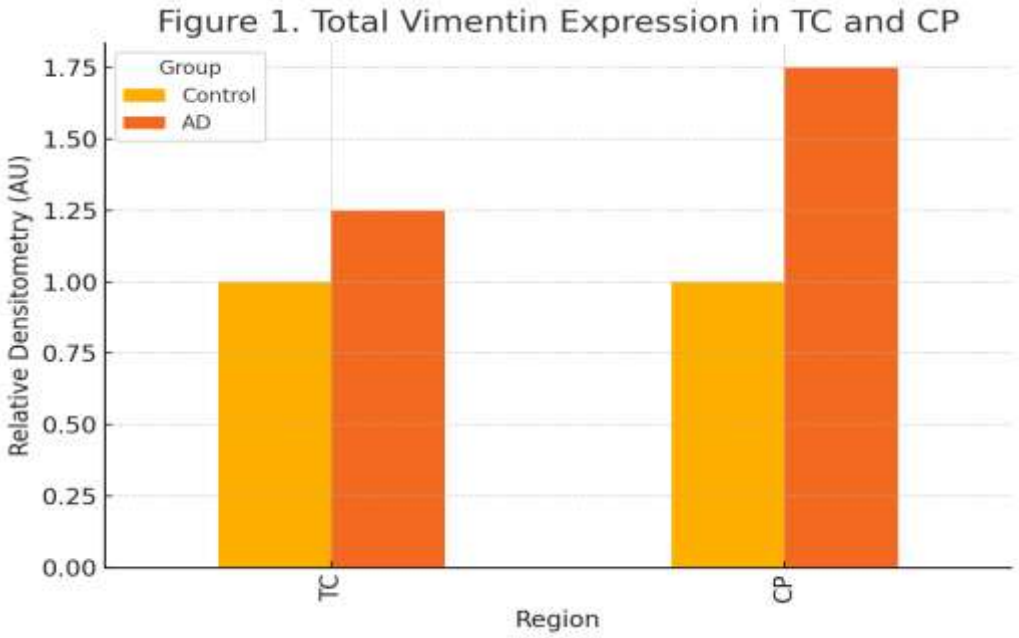


FIGURE 2: PHOSPHO-SER56-VIMENTIN BAND PRESENCE UNDER NORMAL AND HIGH-STRINGENCY CONDITIONS, WITH ELEVATED DETECTION IN AD CP AND AD TC UNDER STRINGENT CONDITIONS

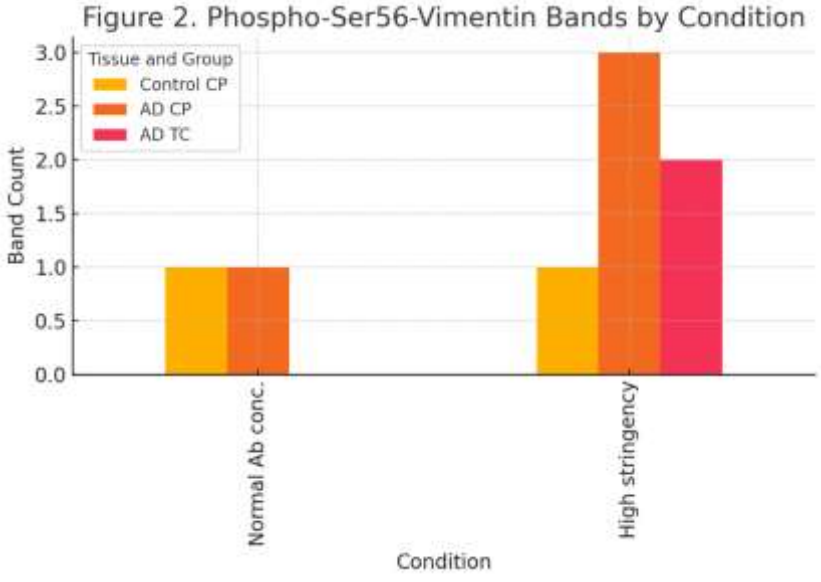
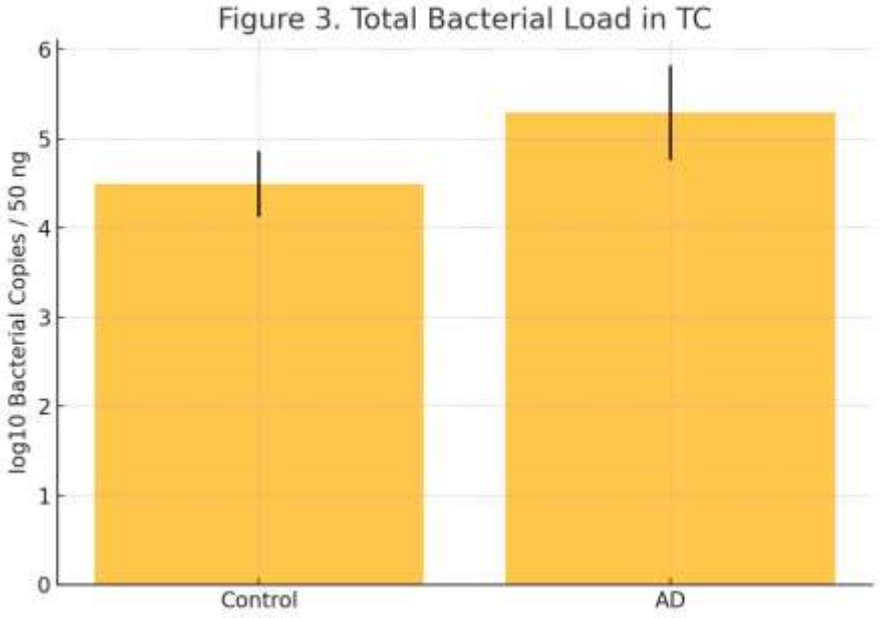


FIGURE 3: LOG10 BACTERIAL COPY NUMBER IN TC, ILLUSTRATING SIGNIFICANTLY HIGHER BACTERIAL LOAD IN AD COMPARED TO CONTROLS



DISCUSSION

Our pilot data substantiate a heightened total bacterial DNA burden in AD temporal cortex, aligning with independent 16S-sequencing studies that describe enriched oral and gut taxa in diseased brains [8,9]. Although the choroid plexus (CP) is anatomically predisposed to microbial ingress, we observed only modest bacterial DNA there, suggesting efficient barrier clearance or preferential colonisation of downstream parenchyma. Experimental infection models show that bacteria exploit CP epithelium transiently before disseminating into periventricular tissue [9,10], which may account for our findings. The trend toward elevated Aβ40 in AD TC mirrors the peptide's vascular tropism and antimicrobial propensity. Nevertheless, lack of correlation with bacterial load implies that Aβ up-regulation may reflect broader inflammatory signalling rather than direct microbial trapping in this small cohort. Future work should incorporate parallel Aβ42 quantification and plaque staging to clarify isoform-specific interactions and regional deposition kinetics. In addition, employing conformation-sensitive dyes or seeding assays could reveal whether bacterial products influence the nucleation competence of soluble Aβ assemblies. Vimentin emerged as a sensitive marker of tissue stress. The protein can be secreted extracellularly to opsonise bacteria and facilitate phagocytosis, while phosphorylation at Ser56 modulates filament disassembly during infection [11]. Our detection of robust phospho-vimentin in CP—even without high bacterial DNA—supports a scenario in which the barrier senses circulating pathogen-associated molecular patterns and mounts a cytoskeletal response. This is consonant with single-cell CP atlases showing age-related expansion of interferon-responsive epithelial subsets. Notably, actin loading controls were universally lower in CP, underscoring intrinsic proteomic differences that warrant normalisation strategies beyond β-actin when comparing barrier with parenchymal tissues. Adoption of total-protein stain membranes or tandem-mass-tag proteomics would yield more reliable cross-tissue comparisons. Methodological strengths include rigorous contamination control, the use of Molzym 16S-free reagents and parallel measurement of host-response markers. Limitations comprise the small sample size, absence of species-level bacterial profiling and reliance on cross-sectional post-mortem tissue that cannot delineate temporal causality. Post-mortem delay and agonal state might differentially affect microbial translocation; controlling for these confounders in larger cohorts is essential. Emerging long-read metagenomics and spatial transcriptomics could resolve whether particular pathogens drive local microglial activation or whether bacterial DNA derives from blood-borne fragments. Moreover, metabolomic screening for microbe-derived lipopolysaccharides, gingipains or short-chain fatty acids may establish functional

links between microbial presence and inflammatory tone. Therapeutically, our data endorse further exploration of antimicrobial strategies. Gingipain inhibitors reduce neuro-degeneration in murine AD models [12], while doxycycline and rifampicin have shown cognitive benefit in small clinical trials. However, barrier-directed approaches targeting CP immune signalling may prove superior to blanket antibiotics, given the low bacterial biomass yet pronounced vimentin activation we observed at this interface. Interventions that stabilise CP tight junctions—such as Wnt agonists or glucagon-like peptide-1 analogues—could conceivably curtail pathogen ingress without perturbing the commensal microbiome. Likewise, repurposing host-directed drugs that dampen vimentin phosphorylation (e.g., Rho-kinase inhibitors) may attenuate downstream inflammasome activation while preserving essential cytoskeletal functions. Finally, our findings have biomarker implications. Circulating extracellular vesicles enriched for phosphorylated vimentin or bacterial 16S fragments might serve as peripheral surrogates of barrier breach, enabling minimally invasive monitoring of therapeutic impact. Longitudinal cerebrospinal-fluid sampling in prodromal individuals, coupled with advanced droplet digital PCR for microbial DNA, could establish whether bacterial signals precede overt cognitive decline. Such studies will be critical for testing the causal rather than correlative role of microbes in AD pathogenesis and for guiding precision antimicrobial or immuno-modulatory interventions.

CONCLUSION

This study demonstrates a significant enrichment of bacterial DNA in the temporal cortex of AD brains and highlights differential host-barrier responses exemplified by vimentin phosphorylation in the choroid plexus. Although Aβ40 levels and bacterial load were not directly correlated, their concurrent elevation in AD supports a complex interplay between microbial stimuli and innate amyloidogenic defences. Our findings reinforce the infectious hypothesis of neuro-inflammation and identify CP cytoskeletal remodelling as a potential biomarker or therapeutic target. Larger, longitudinal studies integrating metagenomics and proteomics are required to confirm causality and to inform precision antimicrobial interventions.

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