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Salivary lactoferrin levels in Down Syndrome: a case-control study

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ABSTRACT

Individuals with Down Syndrome (DS) have a high age-dependent risk of developing Alzheimer's disease (AD). In addition to genetic causes, this high risk involves dysregulated immune-inflammatory system. Low lactoferrin levels, one of the main antimicrobial proteins present in saliva, has been associated with AD. Here, we evaluated whether salivary lactoferrin levels change across the life span of individuals with DS. The study included 152 participants, 72 subjects with DS and 80 euploid individuals, and were divided into those under and over 45 years of age, accordingly with the age-dependent risk of AD. Median of salivary lactoferrin were higher among DS individual, in parallel to salivary total protein, but there were no differences in the ratio of lactoferrin to total protein in saliva between groups. Only DS individuals had higher median salivary lactoferrin levels in the age group under 45 years. Meanwhile non-significant differences were detected for the ratio salivary lactoferrin levels to total salivary protein between groups under 45 years, those levels were lower in DS subjects over 45 years old compared with the age-matched control group. Furthermore, the ratio of salivary lactoferrin levels to total protein in DS was associated with cognitive decline being lower in demented groups compared with mild and moderate cognitive impairment groups. In summary, this study indicates that salivary lactoferrin was dysregulated in DS, with significant lower ratio of salivary lactoferrin levels to total salivary proteins in individuals with DS over 45 years old, a population with a gradually increasing risk of AD.

1. Introduction

Down syndrome (DS), caused by complete or partial triplication of chromosome 21, is the most common chromosomal condition occurring in humans (Antonarakis et al., 2020). Most adults with DS will develop amyloid and tau pathology consistent with Alzheimer disease (AD) by the age of 40–50 (Ballard et al., 2016; Davidson et al., 2018; Fortea et al., 2020; McCarron et al., 2017; Veteleanu et al., 2023; Wiseman et al., 2015).

This increased risk of AD is presumably conferred through genetic predispositions arising from trisomy 21 and amyloid precursor protein (APP) overexpression, but also other genes on chromosome 21 interacting with genes on other chromosomes leading to metabolic dysfunction and dysregulated pathways, including the immune-inflammatory system (Martini et al., 2022). It is now accepted that chronic peripheral inflammation and infections may contribute to AD pathogenesis in DS (Kamer et al., 2016). In combination, these alterations can produce a precarious biological environment that favors the development of AD in people with DS (Flores-Aguilar et al., 2020).

Abnormal responses of the immune system in DS have been linked to increased susceptibility to infections (Ram and Chinen, 2011). For example, it is well established that people with DS have increased prevalence and severity of periodontal diseases (Contaldo et al., 2021). Periodontal diseases can initiate or contribute to the AD pathogenesis through multiple pathways (Schwahn et al., 2022; Yang et al., 2023). Oral bacteria can get into the blood stream, invading the brain, crossing a weakened blood-brain barrier, and contribute to AD pathophysiology (Dominy et al., 2019; Lei et al., 2023). Thus, control of these pathogens by antibacterial approaches could be an alternative to reduce AD development (Plascencia-Villa and Perry, 2020). Alterations in

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immunological parameters have been described in saliva of DS subjects (Chaushu et al., 2002b). It has been reported a severe reduction in the bacterial specific salivary antibodies in DS individuals, resulting a worrying immunodeficiency (Chaushu et al., 2007). Moreover, salivary Ig levels may serve as a predictor of the susceptibility of DS individuals to other infections (Chaushu et al., 2002a, 2003). Additionally, levels of the antimicrobial histatin 5 were significantly decreased in saliva of elderly subjects with DS (Komatsu et al., 2021). Because histatin 5 exhibits potent antifungal activity, this reduction may explain why DS individuals are known to be susceptible to fungal infections, such as oral candidiasis (Maranhão et al., 2020). All these findings point to the high risk of infections in people with DS, attributed, at least in part, to defects of the immune system.

Lactoferrin is one of the major antimicrobial proteins present in saliva with several functions, such as antibacterial, antifungal, antiviral, antiparasitic, anti-inflammatory and immunomodulatory (Berlutti et al., 2011; Kruzel et al., 2017; Valenti and Antonini, 2005). Lactoferrin plays an important role in the host defense against oral pathogens, and it's involved in the control of oral microbiome (Legrand, 2012; Legrand et al., 2005; Lynge Pedersen and Belstrøm, 2019). In this context, it was reported that lactoferrin displayed proteinase inhibitory activity against *P. gingivalis*, significantly inhibiting gingipains (Dashper et al., 2012). However, other studies have also supported that oral pathogens could degrade lactoferrin (Alugupalli and Kalfas, 1996; de Lillo et al., 1996). In addition, Olsen and Singhrao proposed that salivary lactoferrin deficiency may act as an unknown trigger of oral microbial dysbiosis, supporting the concept that low levels of lactoferrin might indicate oral dysbiosis (Olsen and Singhrao, 2021). Reduced salivary lactoferrin levels, along with infection and cholinergic hypothesis, provide a new model to explain AD pathogenesis (Nara et al., 2021). Remarkably, salivary lactoferrin levels are significantly reduced in prodromal AD and AD dementia, as they were associated with the amyloid-PET imaging profile (Antequera et al., 2023; Carro et al., 2017; González-Sánchez et al., 2020). New studies reported reduced levels of salivary lactoferrin in AD patients (Zalewska et al., 2021), and positive correlation of memory impairment and lower levels of salivary lactoferrin in older-aged non-Hispanic white and Black Americans at risk for AD due to parental history (Hammerschlag et al., 2024). However, other study by Gleerup et al. reported no differences between AD patients and controls (Gleerup et al., 2021). Moreover, in the Gleerup study, lactoferrin levels across all groups, including controls, were significantly higher than those reported in other studies (Ramenzoni et al., 2021; Rosa et al., 2017; Wu et al., 2018). Potential explanations for this discrepancy include differences between the studied samples, pre-analytical variables (such as fasting time prior to sample collection), or the assays employed (Gleerup et al., 2021). Additionally, in the Gleerup study, the saliva samples were collected immediately following the lumbar puncture, which raises the possibility that the participants at that time had reduced salivary flow as a consequence of fear, anxiety, or other somatosensory stimuli such as pain (Proctor, 2016). It has been demonstrated that salivary concentrations of lactoferrin and total proteins significantly increase when salivary secretion decreases, as evidenced in patients who have undergone radiotherapy involving the major salivary glands or those with Sjögren's syndrome (Almståhl et al., 2001). Moreover, the association between lactoferrin and Aβ also correlated with poorer memory (Reseco et al., 2021). All these data support the hypothesis that salivary glands dysfunction may be an early event associated with Aβ brain accumulation (Antequera et al., 2021; Bermejo-Pareja et al., 2020). According with this theory, alterations in salivary redox balance associated with chronic inflammation, including reduced lactoferrin concentrations, were also described in AD (Zalewska et al., 2021). Additionally, individuals with DS exhibit high levels of oxidative damage biomarkers in saliva such as superoxide dismutase and malondialdehyde (de Sousa et al., 2015). We therefore hypothesized that lower lactoferrin levels can be observed in individuals with DS, predisposing them to infections and the development of AD. Thus, the aim of this study was to evaluate the potential link between salivary levels of lactoferrin and DS by comparing DS individuals of all ages and non-syndromic age-matched controls.

2. Materials and methods

2.1. Participants

In this case-control study, we included 72 participants with DS (40 females, 32 males, age range 3–66 years, as case group), and 80 age-matched subjects without DS (56 females, 24 males, age range 3–56 years, as control group) (Table 1). These participants were recruited among all individuals with DS who regularly attended special educational or occupational therapy centers in Santiago de Compostela, Lugo, and Madrid (Spain), between December 2022 and November 2023. All participants satisfied the following inclusion criteria: genetically confirmed diagnosis of DS, sufficient degree of collaboration to perform a saliva sampling and availability of an informed consent signed by the participants or their legal guardians. The exclusion criteria were subjects who are taking acetylcholinesterase inhibitors, presence of harmful habits (e.g., smoking), having done moderate physical activity in the last 3 h.

The DS and control subjects were divided into four groups according to age: two groups consist of DS and control subjects under 45 years of age, and the other two groups consisting of DS and control subjects over 45 years of age. This classification was performed accordingly with the age-dependent risk of AD (Ballard et al., 2016; Davidson et al., 2018; Fortea et al., 2020; McCarron et al., 2017; Veteleanu et al., 2023; Wiseman et al., 2015).

The severity of intellectual disability was scored at a consensus meeting between the physician, the neuropsychologist, and the occupational therapist of the center where the participants attended, based on cognitive status, detailed medical history, intellectual disability profiling, and recent life events, as previously described (Handen et al., 2020). During consensus determinations, changes in personality, behavior, and activities of daily living were also considered, following previous recommendations (Smith, 2001). Intellectual disability was

Table 1
Characteristics of participants.

Variable	control	Down Syndrome	Total	Test	P value
N	80	72	152	Chi-sq $\chi 2$ (1) = 0.141	0.708
<45 yearsold	61 (76.25 %)	53 (73.61 %)	114 (75 %)		
≥45 yearsold	19 (23.75 %)	19 (26.39 %)	38 (25 %)		
Age, Mean (DS)	30.30 (15.74)	32.68 (15.77)	31.43 (15.75)	Mann-Whitney W = 2624.500	0.346
<45 years old	24.42 (12.03)	25.60 (12.12)	25.01 (12.07)		
≥45 years old	51.21 (4.23)	52.16 (5.42)	51.68 (4.82)		
Gender (F/M)	56/24	40/32	96/56		
<45 years old	43/18	30/23	73/41		
≥45 years old	14/5	10/9	24/14		
Cognitive decline					
Mild		32 (44.4 %)			
Moderate		24 (33.3 %)			
Severe		10 (13.9 %)			
Dementia		6 (8.3 %)			

Abbreviations: F, female; M, male; *ns*, not significant.

categorized as mild, moderate, severe, or profound according to the Diagnostic and Statistical Manual of Mental Disorders, which considers intellectual functioning (IQ), the individual's adaptive functioning, the level of support required for daily activities, communication skills, social abilities, as well as other factors such as health conditions and environmental influences (Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition).

Severe/profound participants were evaluated with the modified cued recall test (mCRT) Spanish version, which has demonstrated excellent accuracy in detecting AD-related cognitive decline in DS people (Videla et al., 2022). Participants have been classified as demented if there is a history of progressive memory loss, disorientation, and functional decline over a period of at least 1 year (Handen et al., 2020). Classification was based on the individuals' best-ever level of functioning. The information was obtained through family interviews and review of medical or educational records for past assessment results.

The study protocol was approved by the Research Ethics Committee of Santiago-Lugo University (Xunta de Galicia; reference 2018/510), following the standards for medical research in humans recommended by the Declaration of Helsinki. All participants or their legally authorized representative gave written informed consent before enrolment.

This report follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

2.2. Saliva collection

Unstimulated saliva sampleswere collected and processed from all subjects as described previously (Carro et al., 2017). All individuals were asked to avoid eating, drinking, or performing oral hygiene measures for at least 3 h before the sample collection. Saliva samples (~0.5 ml) were kept on ice throughout the collection and stored frozen until processing. Then, saliva samples were centrifuged at 1000 rpm for 10 min at 4 °C and the supernatants were aliquoted in polypropylene tubes with Protease Inhibitor Cocktail (Roche Diagnostics, Mannheim, Germany) and kept on −80 °C until further analytical processing.

2.3. Biochemical analysis

Total protein concentration of saliva samples was analyzed using a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA) according to the manufacturer's instructions.

Levels of lactoferrin in saliva samples were determined by enzyme-linked immunosorbent assays (ELISA) using the Human Lactoferrin ELISA kit (FineTest, Wuhan, China) according to manufacturer's instructions. Saliva samples were first diluted 1:1000 and then run in duplicate. The intra-assay coefficients of variation (CVs) ranged from 4.7 % to 6.0 %, the inter-assay CVs ranged from 4.6 % to 5.3 %, and the lower limit of detection was 0.3 ng/mL according to the manufacturer.

2.4. Statistical analysis

All statistical analyses were performed using R software version 3.4.1 (R Development Core Team, 2017; Vienna, Austria). Baseline characteristics were summarized using standard descriptive statistics. Continuous variables were presented as median [IQR] when normality assumptions were not met, and as mean [SD] for normally distributed data. The chi-squared test was used to assess differences between categorical variables, while the Mann–Whitney *U* test was applied for comparisons of non-normally distributed continuous variables. One-way analysis of variance (ANOVA) was used for multigroup comparisons of normally distributed data, and the Kruskal-Wallis test was used for non-normally distributed data, followed by the Bonferroni test for pairwise comparisons. Differences in the median scores of salivary markers among age groups were analyzed using the Scheirer-Ray-Hare test. To examine changes in salivary markers across age, we fitted linear regression models and applied locally estimated scatterplot smoothing (LOESS) regression for each study group. The correlation between concentrations of salivary total protein and lactoferrin was assessed using the Pearson correlation test. A p-value <0.05 was considered statistically significant for all tests.

3. Results

3.1. Study population

The Table 1 displays baseline demographic and cognitive score data for the study. The results confirm that DS cases and controls have similar sex and age distribution. Of the 72 DS subjects, 32 individuals (44.4 %) presented mild cognitive decline, 24 (33.3 %) moderate, 10 (13.9 %) severe, and 6 (8.3 %) suffered from dementia.

3.2. Concentrations of lactoferrin and total protein in the saliva of DS and control subjects

We first analyzed salivary levels of lactoferrin measured in the whole study groups. The median lactoferrin levels were higher in DS cases (7.43 μg/ml [95 % CI; IQR, 4.72–10.86 μg/ml]) compared to those observed in controls (4.44 μg/ml [95 % CI; IQR,2.98–7.10 μg/ml]) ($p = 0.001$; Fig. 1A and Table S1). Statistical analysis also showed that DS group had significantly higher median salivary protein concentration (265.68 μg/ml [95 % CI; IQR, 169.61–410.05 μg/ml]) than the control group (183.43 μg/ml [95 % CI; IQR, 90.61–356.39 μg/ml]) ($p = 0.0021$; Fig. 1B and Table S1). Furthermore, there was a positive correlation between salivary lactoferrin levels and total salivary protein concentration only in the DS group (Pearson correlation $r = 0.34$; $p = 0.003$; Fig. 1C). It is relevant to note that we didn't find significant differences in the mean total salivary protein concentration between mild cognitive impairment, AD patients and control subjects (Carro et al., 2017).

Thus, when the ratio of salivary lactoferrin content to total protein was compared between DS and control groups, no significant differences were observed in the whole cohort (Fig. 1D and Table 1).

We also analyzed the association between each of the main variables (salivary lactoferrin levels, total salivary protein levels, and ratio of salivary lactoferrin levels to total salivary protein) with respect to the study groups and sex, as well as the interaction between both. The results showed no significant differences between males and females (Table S2), despite of the differences in prevalence of AD in both sex.

3.3. Age differences in lactoferrin and total protein levels in the saliva of DS and control subjects

We next analyzed the association between each of the main variables with respect to the study groups and age with a threshold of 45 years. Statistical analysis showed that, in subjects under 45 years of age, the mean salivary concentration of lactoferrin was significantly higher in DS subjects than in the age-matched control group ($p < 0.001$; Fig. 2A and Table S3). Additionally, salivary lactoferrin levels also rose with age with significant differences in the control group between under and over 45 years of age ($p < 0.001$; Fig. 2A and Table S3). Lineal regression analysis also revealed these differences between study groups under 45 years of age, meanwhile the LOESS regression shows a downward trend in lactoferrin values from the age of 45 years in subjects with DS compared to the continued rise in controls (Fig. 2B, and Table S4).

The total salivary protein concentration was significantly higher in DS subjects under 45 years of age compared to the age-matched control group, meanwhile there were no significant differences between study groups over 45 years of age ($p < 0.001$; Fig. 2C and Table S5). The regression curves of the linear model also revealed these differences between study groups under 45 years of age. In the LOESS curve, a similar upward trend was observed in total salivary protein values in both groups until 45 years of age, and from then on, the trends became similar (Fig. 2D, and Table S6).

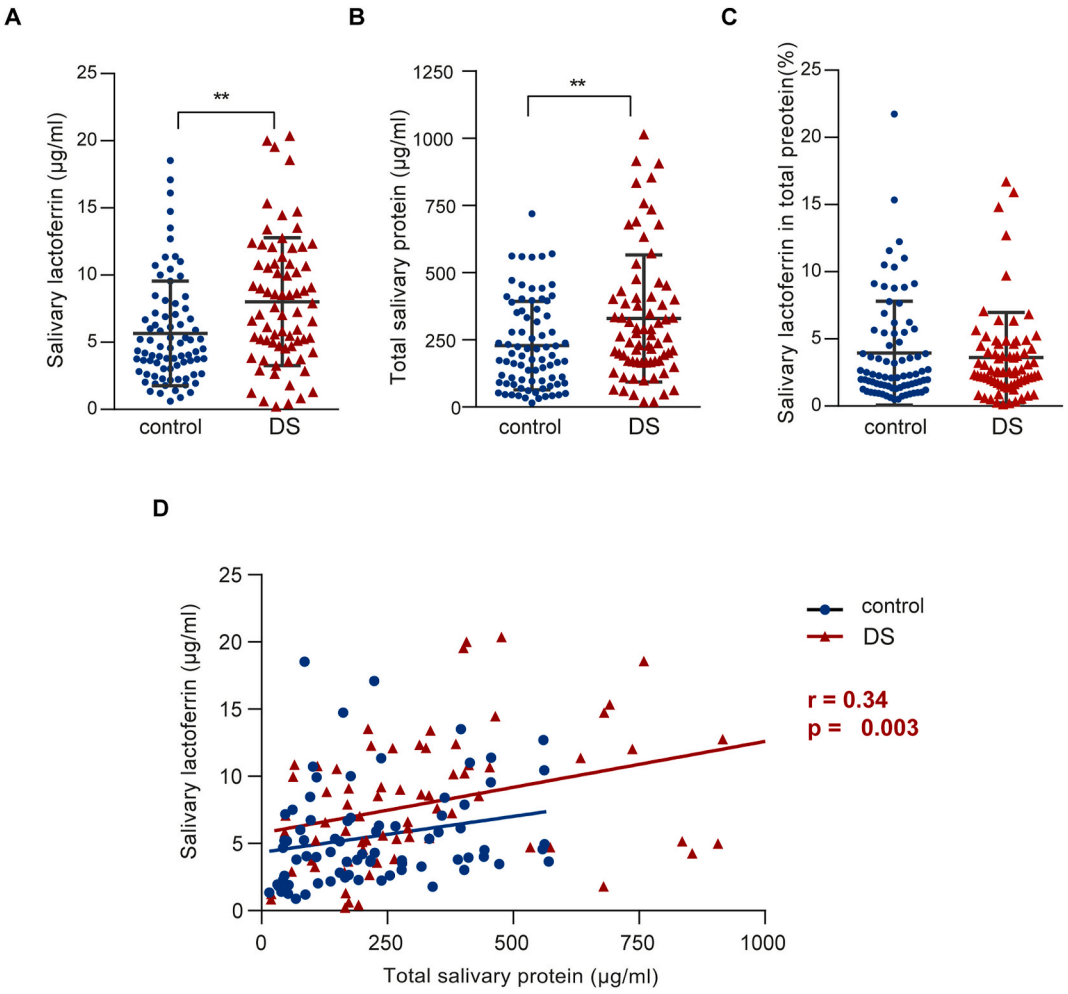

Fig. 1. Concentrations of lactoferrin and total protein in the saliva of Down Syndrome (DS) individuals compared with control subjects. Comparison of salivary lactoferrin (A), and total protein (B) concentrations, and salivary lactoferrin levels in total salivary proteins (D) between the DS and normal groups. (C) Correlation between salivary lactoferrin and total salivary proteins in DS and control groups. (n = 72 DS, 80 control). $^{**}p < 0.01$, $^{***}p < 0.001$.

As salivary levels of lactoferrin were correlated with the total salivary protein concentration, lactoferrin levels were normalized to the total protein concentration in saliva. Statistical analysis revealed that in subjects over 45 years of age, the ratio of salivary lactoferrin levels to total protein in DS subjects was lower than in controls ($p = 0.013$; Fig. 2E and Table S7). The regression curves of the linear model LOESS curves only tended to diverge between DS and control groups over 45 years of age (Fig. 2F).

3.4. Association of cognitive decline with age and lactoferrin and total protein levels in the saliva of DS subjects

We also analyzed the prevalence of cognitive decline with age in DS subjects based on the severity of intellectual disability of each participant. The changes in the cognitive decline performance with age in DS subjects is shown in Fig. 3A. Statistical analysis suggests that there was a significant difference in ages between the four cognitive deficit groups ($\chi 2 = 18.700$; $p < 0.001$; Fig. 3A). As expected, the cognitive decline scores increased with age, and DS individuals with severe cognitive decline and dementia were significantly older than those with moderate cognitive decline (Fig. 3A and Table S8).

We next compared salivary lactoferrin and total protein concentrations across cognitive decline scores. The means of salivary lactoferrin concentration or salivary protein concentration did not differ by cognitive decline (Fig. 3B and C). However, the ratio of salivary lactoferrin levels to total protein in DS subjects varied depending on the degree of cognitive decline being lower in demented group comparing with moderate cognitive impairment group ($\chi 2 = 9.958$; $p = 0.019$; Fig. 3D and Table S9).

An analysis was carried out using a linear model to determine the joint influence of age and cognitive deficit. Linear regression model fitting revealed that the joint influence of age and cognitive deficit in the demented group significantly affected the ratio of salivary lactoferrin levels to total protein in DS subjects ($p = 0.008$; Fig. 3E). This ratio decreases exponentially with age in the DS demented group, approximately 2.5 % for each additional year of age.

4. Discussion

Reduced salivary levels of lactoferrin have been reported and suggested as potential biomarker of AD (Antequera et al., 2023; Carro et al., 2017; González-Sánchez et al., 2020). The essential roles played by lactoferrin point to their levels as a key target of vulnerability to neurodegeneration (Abdelhamid et al., 2020; Eker et al., 2023; Liu et al., 2020; Xu et al., 2019; Yong et al., 2023). In the present study, we have analyzed the salivary lactoferrin levels of DS and control individuals at different ages. To our knowledge, this is the first population-based cohort study exploring age-related changes of salivary lactoferrin levels in people with DS.

Our results showed that there are differences in the salivary protein

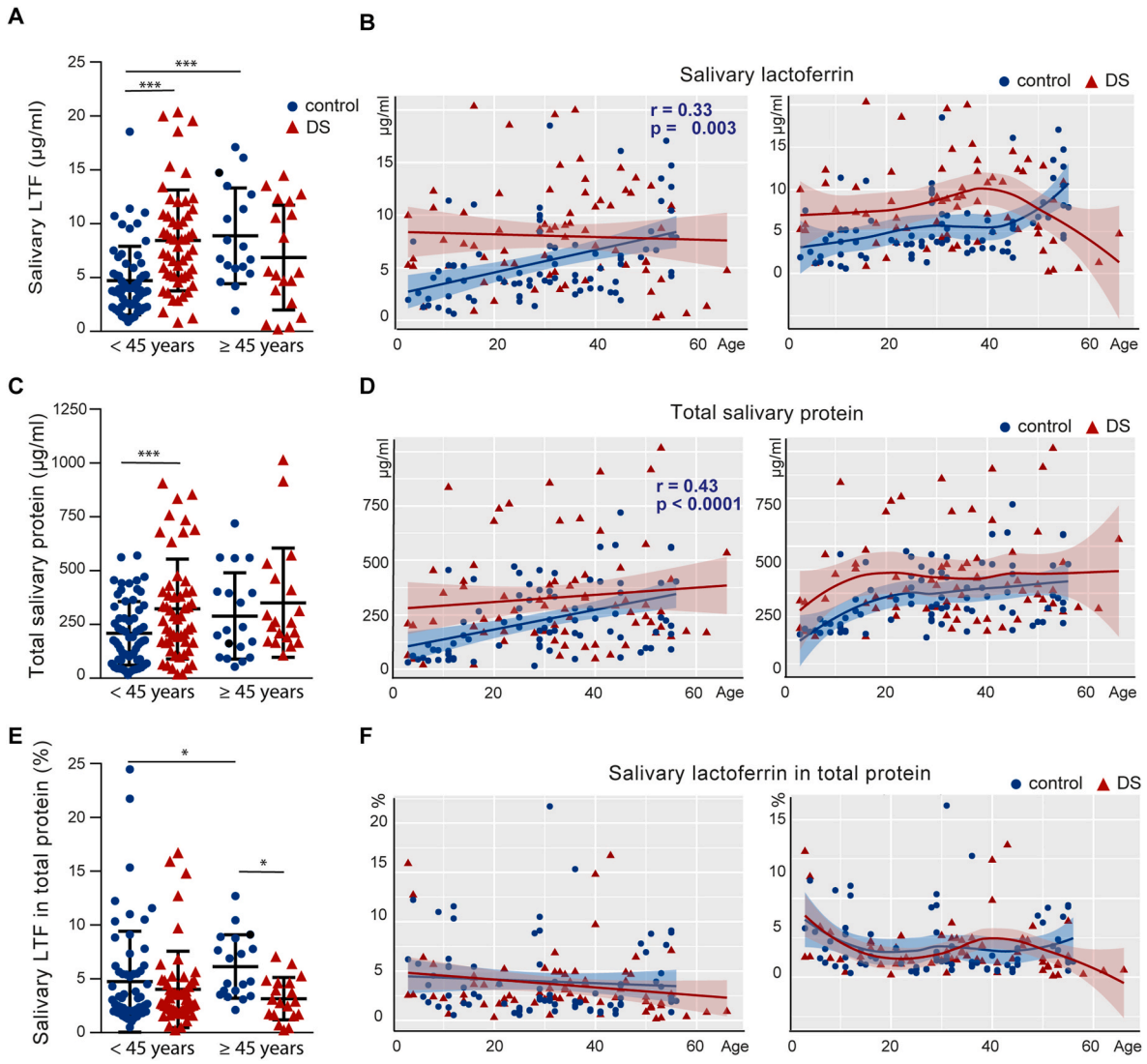

Fig. 2. Changes with age in salivary concentration levels by study groups. Scatter plots represent the median concentrations of salivary lactoferrin (A), total salivary protein (C), and the ratio of salivary lactoferrin levels in total salivary proteins (E) in DS and control groups subdivided according to age (under and over 45 years of age). Longitudinal changes with age in salivary lactoferrin (B), total salivary protein (D), and the ratio of salivary lactoferrin levels in total salivary proteins (F) using regression curves of the linear (left panels) and LOESS (right panels) models for each group and 95 % CI, representing the evolution of age-related changes. LTF: lactoferrin. (n = 72 DS, 80 control). $^{*}p < 0.05$, $^{***}p < 0.001$.

concentration between people with and without DS. In the whole cohort, we found that salivary lactoferrin levels were significantly higher (~30 %) among people with DS than those observed in the control group. Moreover, total protein concentration was more than 68 % higher in the saliva of DS group compared to the control group. This is in line with previous data showing that total protein concentration in DS saliva was significantly higher compared to that observed in the saliva of control subjects (Komatsu et al., 2021; Siqueira and Nicolau, 2002; Yarat et al., 1999). In the present study, we found a correlation between salivary lactoferrin and total protein concentrations in the entire cohort but also in each of the groups studied. Therefore, when lactoferrin levels were adjusted to the total protein concentration in saliva, no statistically significant differences were seen between the groups.

We found a clear age dependency pattern of salivary lactoferrin levels, different between DS and control subjects. In saliva samples from children and young adults under 45 years, the lactoferrin concentrations were significantly higher in the DS group than in the control group, meanwhile it was similar in both study groups over 45 years. If we analyze the effect of age in each of the study groups, lactoferrin levels increased with age among healthy individuals while remaining stable in the DS group. Under 45 years of age, total salivary protein concentrations were also higher in DS individuals compared to age-matched healthy subjects. Taken together, our findings indicate that DS individuals over 45 years have lower salivary lactoferrin levels, related to the amount of total protein, compared to age-matched controls. These results are consistent with findings reporting reduced salivary levels of histatin 5 in elderly individuals with DS (Komatsu et al., 2021). We propose that deficits in the salivary levels of these antimicrobial proteins would indicate that immune response is attenuated in DS individual aged over 45 years, a population group at higher risk to AD developing (Ballard et al., 2016; Davidson et al., 2018; Fortea et al., 2020; McCarron et al., 2017; Veteleanu et al., 2023; Wiseman et al., 2015).

Healthy infants have an adaptive immune system that matures with age, to establish defense mechanisms against foreign structures such as viral or bacterial pathogens (Pieren et al., 2022; Simon et al., 2015). Notably, in the general population, salivary lactoferrin levels are lower in children than in young adults (Tenovuo et al., 1986), reaching the highest levels in the middle adult stage to end up descending in advanced age adults and elderly (Bartolome et al., 2021; Shugars et al., 2001). This is in line with our data, where control individuals under 20

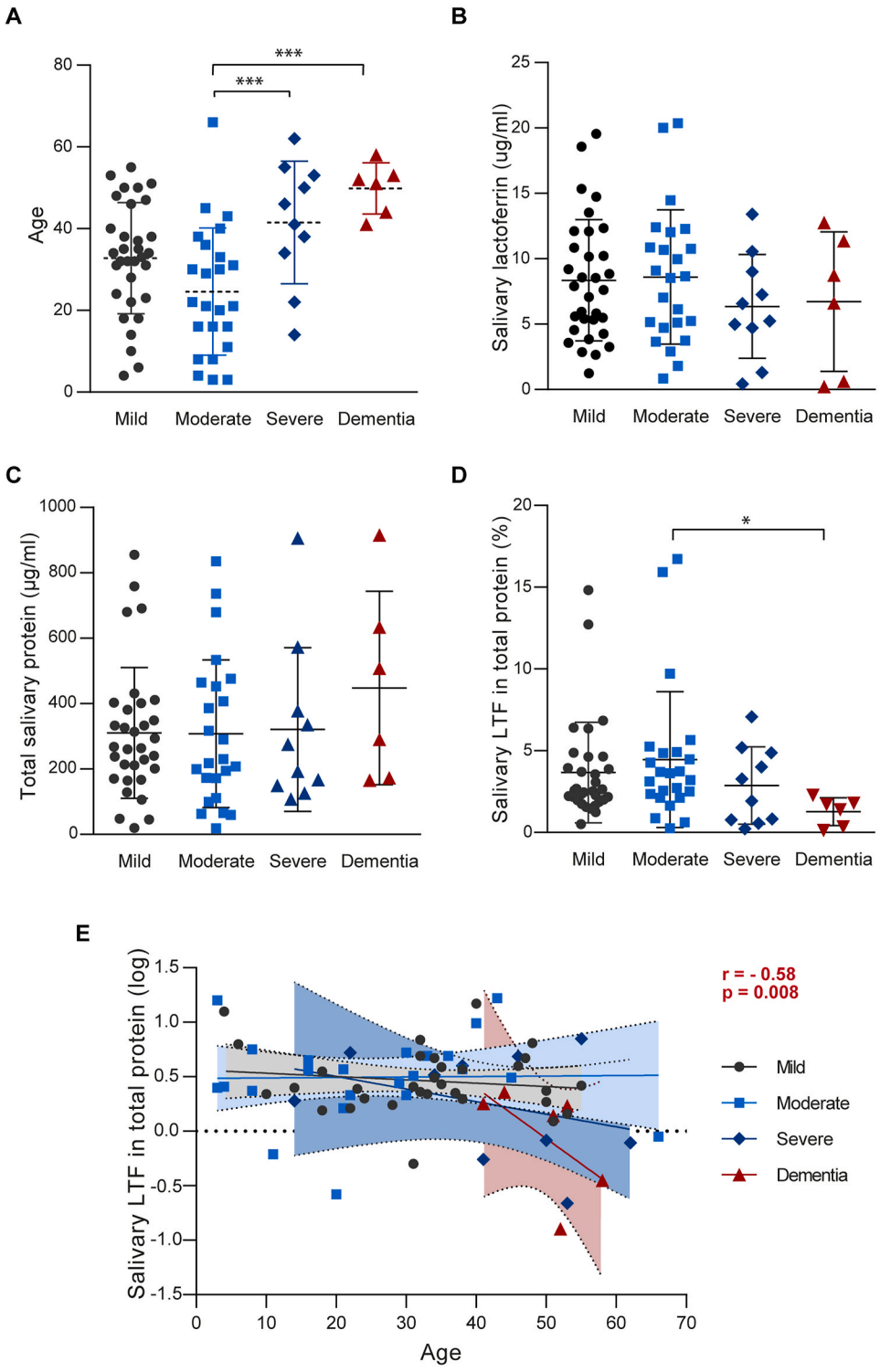

Fig. 3. Association of salivary concentrations with cognitive decline in DS subjects. (A) Scatter plots of the median age in the DS group by cognitive decline. (B–D) Scatter plots of the median concentrations of salivary lactoferrin (B), total protein (C), and lactoferrin in total protein (D) in the DS groups according with their cognitive decline score. (E) Longitudinal changes in the ratio of salivary lactoferrin in total protein according to cognitive decline in DS subjects. Colored lines and bands denote the lineal regression and its 95 % CI. (n = 72 DS [32 with mild cognitive impairment, 24 with moderate, 10 with severe, 6 with dementia). $*p < 0.05$, $***p < 0.001$.

years old had an average of lactoferrin levels in saliva lower than younger individuals (20–45 years old). In our present study, DS subjects under 45 years of age had increased salivary lactoferrin levels than control subjects at the same age, however when they were normalized to the total protein concentration in saliva, there was no differences in the resulting ratio. Moreover, while the ratio of salivary lactoferrin levels to total protein increased with age in control population, it remained unchanged in DS group, suggesting that some immunological mechanisms could be altered. In fact, in DS subjects over 45 years of age this ratio is lower than in age-matched controls. Our findings suggest that salivary

defenses against microbial infections may not have been fully developed throughout their lifetime. Reduced ratio of salivary lactoferrin levels to total protein in DS subjects at older ages can facilitate proliferation of oral pathogens, acting as a trigger for oral dysbiosis (Kruzel et al., 2017), and the development of chronic infection (Olsen and Singhrao, 2021).

It is well known that individuals with DS exhibit a higher risk of suffering infectious diseases compared to the general population, including periodontal diseases (Abanto et al., 2011; Contaldo et al., 2021). Among the oldest known immune defense molecules, antimicrobial proteins/peptides control oral microorganisms (Gorr and Abdolhosseini, 2011; Johnstone and Herzberg, 2022). Specifically, lactoferrin is a first line defense protein for protection against microbial infections, contributing to the maintenance of oral eubiosis (Kruzel et al., 2017; Lynge Pedersen and Belstrøm, 2019). We and others propose that low salivary lactoferrin content might facilitate for oral dysbiosis to proceed freely, and the expansion of pathogens or their inflammatory products to the brain (Bermejo-Pareja et al., 2020; Municio and Carro, 2023; Olsen and Singhrao, 2021). In humans, dysbiosis of the oral subgingival microbiome has been associated with cerebrospinal fluid (CSF) evidence of the AD-signature pathology, which includes Aβ and tauopathy (Kamer et al., 2021). It is remarkable that a special vulnerability to infections affects individuals with DS after the age of 50 years (Guffroy et al., 2019). From that age, rates of dementia reach up 55–70 % (Ballard et al., 2016). This is consistent with the effect of dysregulated immune system, affecting both innate and adaptive immunity, in the increased risk of AD associated with DS (Martini et al., 2022). Taken together, this is in line with our previous hypothesis that low salivary lactoferrin concentrations might represent a decline in the oral defensive protection, exacerbating the risk of AD (Bermejo-Pareja et al., 2020). And this theory might be also applied in DS, as the ratio of salivary lactoferrin levels to total protein is particularly lower in DS subjects over 45 years of age.

Recently, disturbances in iron homeostasis linked to increased cytokine expression and hepcidin, a hormone that regulates systemic iron homeostasis, were described in people with DS and AD, suggesting shared mechanisms between increased susceptibility to infections and neurodegeneration (Raha-Chowdhury et al., 2021; Raha et al., 2021). Iron dyshomeostasis and decreased levels of transferrin in DS, lead to upregulation of neurotoxicity mechanisms (Barone et al., 2018). Lactoferrin plays a key role in iron homeostasis, with an iron sequestration mechanism, resulting in a decrease in free iron availability that can limit the growth and pathogenicity of invasive microbial pathogens, providing an important means of host defense (Bartolomé et al., 2022; Rosa et al., 2017; Velliyagounder et al., 2018). We cannot exclude the role of lactoferrin in the iron dyshomeostasis in DS, as iron overload facilitating the growth of microbial pathogens (Fig. 4).

Dysfunction of salivary glands in DS has been also reported, including changes in saliva composition or even absence of salivary glands (Chaushu et al., 2002b, 2007; Komatsu et al., 2021; Odeh et al., 2013). Since lactoferrin in saliva is mainly secreted by salivary glands, the decrease in lactoferrin levels in DS saliva may be explained by salivary gland dysfunction. Moreover, hypothalamic abnormalities in DS, such as neuronal loss (Wisniewski and Bobinski, 1991), or circadian-related disturbances (Bassell et al., 2015; Fernandez et al., 2017; Leng et al., 2019) were reported. As salivary gland secretion is under hypothalamic control (Proctor and Carpenter, 2007, 2014), hypothalamic alterations in DS could lead to salivary glands deregulation, similarly to that described in AD models (Antequera et al., 2021).

Our study also showed that salivary lactoferrin levels are strongly associated with cognitive decline in DS. These findings may have important implications for differentiating dementia-related cognitive decline from intellectual disability in people with DS. This is in line with recent studies highlighting the need for accessible and non-invasive biomarkers for detecting AD pathophysiological processes in DS individuals and predicting the onset of neurodegenerative cognitive decline (Carmona-Iragui et al., 2021; Grasso et al., 2024; Pentz et al., 2021; Snyder et al., 2020). Taken together, our results suggest that reduced salivary lactoferrin levels to total protein might be a potential biomarker able to predict the following cognitive decline in DS subjects.

This study is not exempt from certain limitations and future research should include improvements. The sample size is limited, to include a larger cohort would allow to study more potential association with clinical and demographic parameters, including more neurological and neuropsychological assessments to perfectly discriminate between impairment due to AD dementia and intellectual disability because of DS. Increase the sub-analysis by age. Lastly, longitudinal studies will allow longitudinal cognitive assessments, including prodromal, AD dementia and accuracy value of salivary lactoferrin in people with DS. Moreover, additional studies using larger cohorts would allow us to explore whether lactoferrin levels in other fluids, such as blood, vary in

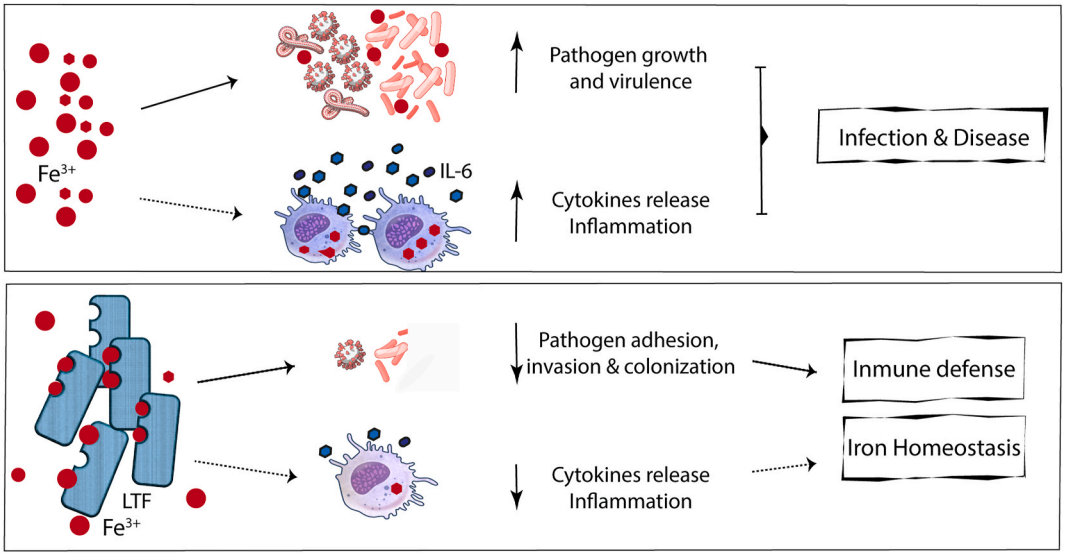

Fig. 4. Diagram on the role of the iron dyshomeostasis. Upper panel: The availability of free iron (Fe^{3+}) favors the growth of pathogens and increases their virulence. It also affects immune system cells, which release more pro-inflammatory cytokines, triggering an inflammatory process. Lower panel: Each molecule of lactoferrin is capable of capturing two Fe^{3+}, this decreases its availability to pathogenic microorganisms, limiting their growth, adhesion, invasion, and colonization. It also reduces the inflammatory process.

patients with DS.

5. Conclusions

In summary, this study found that salivary lactoferrin was dysregulated in DS, with significant lower ratio of salivary lactoferrin content to total protein in individuals with DS over 45 years old, a population with a gradually increasing risk of AD, suggesting that salivary lactoferrin can reflect an immune dysregulation state. These findings support the need for assisting DS individuals to prevent or delay AD-onset in future clinical trials.

CRediT authorship contribution statement

Desireé Antequera: Methodology, Investigation, Formal analysis. **Lucía Sande:** Methodology, Investigation, Formal analysis. **Eliane García Mato:** Methodology, Investigation, Formal analysis. **Deborah Romualdi:** Methodology, Investigation. **Laura Carrero:** Methodology, Investigation. **Cristina Municio:** Methodology, Investigation. **Pedro Diz:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Eva Carro:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Eva Carro reports administrative support was provided by Carlos III Health Institute. Reports a relationship with that includes:. Has patent pending to. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2025.100999.

Data availability

Data will be made available on request.

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