Neurobiological Effects of Lipoid Proteinosis: A Study on Phosphorylated Tau, S100B, NSE, NEFL, and GFAP

Lipoid Proteinozisin Nörobiyolojik Etkileri: Fosforile Tau, S100B, NSE, NEFL ve GFAP Üzerine Bir Araştırma

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Abstract

Background: Lipoid Proteinosis (LP), also termed Urbach-Wiethe disease, is an enigmatic genodermatosis marked by the systemic deposition of hyaline material. With its etiology rooted in ECM1 mutations, LP’s neuro-pathological spectrum has been hypothesized to involve an array of neurodegenerative biomarkers, underscoring a potential for substantial neurobiological implications. This study endeavored to elucidate the serum concentrations of neurodegenerative biomarkers—phosphorylated Tau (pMAPT), S100B, Neuron-Specific Enolase (NSE), Neurofilament Light Chain (NEFL), and Glial Fibrillary Acidic Protein (GFAP)—in LP patients, seeking to establish their diagnostic utility for the condition.

Materials and Methods: Fifteen LP patients and 15 matched healthy controls were enrolled. Serum levels of the biomarkers were quantified using ELISA, and their predictive power was assessed through binary logistic regression and Receiver Operating Characteristic (ROC) analysis.

Results: Elevated serum levels of NSE, NEFL, and GFAP were observed in LP subjects relative to healthy counterparts, reaching statistical significance ($p<0.05$). In contrast, pMAPT and S100B levels did not differ appreciably. GFAP is considered a predictive marker for LP with an area under the curve (AUC) value of 0.813 and a 95% confidence interval (CI) of 0.658-0.968 ($p=0.003$).

Conclusions: The study underscores a distinctive neurodegenerative profile in LP, with NSE, NEFL, and GFAP concentrations significantly amplified. These biomarkers, particularly GFAP, may represent novel indicators for LP, offering prospective biomarker-based diagnostic strategies. The insights garnered herein pave the way for advanced understanding and clinical management of LP, delineating a novel avenue for future high-impact research.

Key Words: Lipoid Proteinosis, Neurodegeneration, Biomarkers, Diagnostic Neurology

Öz

Amaç: Urbach-Wiethe hastalığı olarak da adlandırılan Lipoid Proteinosis (LP), hiyalin materyalinin sistemik olarak birikmesiyle belirginleşen bir genodermatozdur. Etiyolojisi ECM1 mutasyonlarına dayanan LP’nin nöropatolojik spektrumunun, önemli nörobiyolojik etkilerini potansiyeline olan bir distant nörodejeneratif biyobelirteç içi olduğu varsayılır. Bu çalışma, LP hastalarında nörodejeneratif biyobelirteçlerin (fosforile Tau (pMAPT), S100B, Nöron Spesifik Enolaz (NSE), Neurofilament Hafif Zincir (NEFL) ve Glial Fibriller Asidik Protein (GFAP) ) serum konansyonlarını ölçmeyi ve bu belirteçlerin tanısal potansiyellerini belirlemeyi amaçladık.

Materyal ve Metod: 15 LP hasta ve 15 sağlıklı kontrol çalışmaya dahil edildi. Nörodejeneratif biyobelirteçlerin serum seviyeleri ELISA testleri kullanılarak ölçülüp ve prediktif güçleri iki lojistik regresyon ve Receiver Operating Characteristic (ROC) analizi yoluyla değerlendirildi.

Bulgular: LP hastalarında sağlıklı kontrolere göre NSE, NEFL ve GFAP serum düzeyleri daha yüksekti ve bu artışın istatistiksel olarak anlamılı olduğu bulundu ($p<0.05$). Buna karşılık, pMAPT ve S100B seviyelerinde anlamalı farklılık gözlenmedi. GFAP’nin 0.813’ü eğrili altında kalan alan (AUC) değeri ve 0.658-0.968’lik %95 güven aralığı (CI) ile LP için prediktif bir belirli olabildiğine dair bir sonucu verdi ($p=0.003$).

Sonuç: Bu sonuçlar NSE, NEFL ve GFAP konansyonlarının LP’de önemli ölçüde artışını göstermektedir ve LP’de belirgin bir nörodejeneratif profilin altını çizmektedir. Bu biyobelirteçler, özellikle GFAP, LP için yeni bir indikatör olabilir ve ileriye yönelik biyobelirteç bazı tanı stratejileri sunabilir. Bu çalışmada elde edilen bilgiler, gelekteki yüksek etkili araştırmalar için yeni bir yol çizer LP’nin anlaşıması ve klinik yönetiminde yolküstünü olabilir.

Anahtar Kelimeler: Lipoid Proteinosis, Nörodejenerasyon, Biyobelirteçler, Tansal Nöroloji

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Introduction
Lipoid proteinosis (LP), also known as Urbach-Wiethe disease, is a rare autosomal recessive genodermatosis marked by the accumulation of amorphous hyaline material in numerous parts of the body, including the skin, mucous membranes, brain, and internal organs (1). LP often manifests in the first few months of life and is indicated by a harsh, feeble scream brought on by laryngeal infiltration (2). The disease exhibits a wide range of clinical manifestations, including varying levels of skin scarring, hoarseness, respiratory distress, and, in certain instances, neurological abnormalities like temporal lobe epilepsy (3). Bilateral amygdalae calcification characterizes LP and can impact various gastrointestinal organs, displaying consistent macroscopic and microscopic lesions (4,5). It has the potential to result in severe medical complications, including acute respiratory distress and seizures (6). The symptoms of the disease are heterogeneous and may vary among patients due to involvement of different organ systems. The disorder results from deleterious mutations in the glycoprotein extracellular matrix protein 1 (ECM1) (7). The ECM1 protein plays a critical role in various biological processes by regulating the structural integrity of the extracellular matrix and cell-matrix interactions, including angiogenesis, cell adhesion, and cell differentiation. Mutations in ECM1 that cause abnormal protein expression may lead to protein accumulation and calcification in organs in the process (8). LP is distinguished by the pathognomonic brain imaging finding of bilateral intracranial calcification in the temporal lobes (9). Calcifications in the brain are frequently observed in the amygdala region but can also extend to areas such as the hippocampus, parahippocampal gyrus, and striatum. It has been reported that calcifications in the temporal lobes and hippocampus are associated with neurological, psychiatric, and cognitive disorders (10). The neurological manifestations of lipoid proteinosis can vary in type and severity, including conditions such as epilepsy, dystonia, progressive neuropsychiatric disorders (such as memory loss, cognitive impairments, hallucinations, and schizophrenia-like disorders), as well as spontaneous central nervous system hemorrhages. (11,12) Literature reports intracranial calcifications and abnormal protein accumulations in patients with LP. These structural changes can lead to both structural and functional alterations in the brain, resulting in a range of neurological and psychiatric symptoms (10). However, there is still no clear evidence regarding whether these changes directly cause neuronal damage or contribute to neurodegenerative processes.

Neurodegenerative biomarkers, including phosphorylated Microtubule-Associated Protein Tau (pMAPT), S100 Calcium Binding Protein B (S100B), Neuron-Specific Enolase (NSE), Neurofilament Light Chain (NEFL), and Glial Fibrillary Acidic Protein (GFAP), have been studied extensively in the context of neurodegenerative diseases. pMAPT is a critical biomarker associated with neurodegenerative disorders, particularly Alzheimer’s disease, where abnormal phosphorylation leads to microtubule destabilization and the formation of neurofibrillary tangles (13,14). S100B is another important biomarker, often elevated after brain injury, reflecting astroglial activation and potential neuronal damage (15,16). NSE is a well-established biomarker associated with neuronal damage, often used to assess brain injury and neurodegenerative diseases. Studies have shown that NSE is expressed in neurons and microglia after brain injury, indicating its association with neuronal damage (17). NEFL is a component of the neuronal cytoskeleton and is released into the cerebrospinal fluid following neuronal damage, making it a potential biomarker for neurodegenerative diseases (18,19). GFAP is a well-established astroglial activation biomarker associated with various neurological conditions, including neurodegenerative diseases, brain injury, and autoimmune disorders (20,21).

While calcifications and protein accumulations in lipid proteinosis are commonly associated with neurological and psychiatric symptoms, the effects of these changes on neuronal damage and neurodegeneration remain uncertain. This study aims to investigate the neurobiological effects of LP on key neurodegenerative biomarkers (phosphorylated Tau, S100B, NSE, NEFL, and GFAP), to understand how the disease modifies these markers and influences neurodegenerative pathways. The goal is to enhance our understanding of Lipoid Proteinosis’s neurological aspects and its pathophysiological mechanisms, offering new insights that could lead to the development of innovative therapeutic strategies targeting its neurodegenerative effects.

Materials and Methods
Study Population and Design
This study comprised 15 patients clinically and histopathologically diagnosed with LP, along with 15 gender- and age-matched healthy controls. Exclusion criteria for both groups included a history of alcohol consumption or smoking, presence of infectious diseases, acute or chronic systemic conditions, and recent intake of drugs or vitamins. The study protocol adhered to the ethical standards established by the Declaration of Helsinki and Good Clinical Practice guidelines, and received approval from the Clinical Research Ethics Committee of Harran University (HRU/23.24.26). Informed consent was obtained from all participants or their legal guardians.

Sample Collection and Processing
Blood specimens were procured post an obligatory fasting period of eight hours, a protocol to ensure metabolic homogeneity. Employing biochemical tubes for collection, the samples were then subjected to centrifugation at 1500 g.
for a duration of 10 minutes, a step critical for serum separation. The extracted serum was subsequently stored at -86°C, a measure imperative for maintaining the analytical integrity of the neurodegenerative biomarkers.

**Neurodegenerative Biomarker Analysis**

The study focused on the quantification of neurodegenerative biomarkers, namely pMAPT, S100B, NSE, NEFL, and GFAP. The quantification of these biomarkers in serum samples was performed utilizing specific enzyme-linked immunosorbent assay (ELISA) kits provided by Elabscience, with kit references: pMAPT (cat. no. E-EL-H5314), S100B (cat. no. E-EL-H1297), NSE (cat. no. E-EL-H1047), NEFL (cat. no. E-EL-H0741), and GFAP (cat. no. E-EL-H6093). The methodology adhered strictly to the assay protocols furnished by the manufacturer. Serum samples and standards were loaded in duplicate into the ELISA plates according to manufacturer instructions. Biotinylated detection antibodies and Avidin-HRP conjugate was added, followed by washing and adding a substrate solution. Then the optical density was measured with a microplate reader at a wavelength of 450 nm and biomarker concentrations were calculated by comparing the optical density of the samples with the standard curve. Measurements were executed utilizing a microplate reader system (ThermoFisher Scientific Varioskan™ LUX multimode microplate reader, USA) facilitating precise and reliable quantification. S100B, NEFL, pMAPT and GFAP levels were expressed as pg/mL, NSE level as ng/mL.

**Statistical Analyses**

For the purpose of statistical evaluation, SPSS software, version 25.0 (SPSS Inc., Chicago, IL, USA), was utilized. The Pearson’s chi-square test was employed to analyze categorical variables. The normality of the data procured in this study was assessed using the Shapiro-Wilk or Kolmogorov-Smirnov test, as appropriate. Continuous variables adhering to a normal distribution were compared using the Student’s t-test. In contrast, the Mann-Whitney U-test was applied for comparative analysis for variables that deviated from normal distribution. Receiver Operator Characteristic (ROC) analysis was employed to determine the diagnostic precision of biomarkers associated with neurodegenerative conditions. Hypotheses were modeled using binary logistic regression. Data conforming to normal distribution were expressed as mean ± standard deviation, while data not conforming to normal distribution were presented as median [interquartile range]. A p-value of less than 0.05 was considered statistically significant.

**Results**

The demographic characteristics of the participants, including both patients diagnosed with LP and individuals in the control group, are summarized in Table 1. There were no statistically significant differences between the LP group and the control group in terms of gender distribution (p=0.269), age (p=0.677), and body mass index (BMI) (p=0.770).

<table>
<thead>
<tr>
<th>Table 1. Sociodemographic characteristics of case and control groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lipid Proteinosis (n=15)</strong></td>
</tr>
<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td>Gender (F/M)</td>
</tr>
<tr>
<td>Age (Years)</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
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</tbody>
</table>

Mean ± Standart Deviation, BMI: body mass index.

<table>
<thead>
<tr>
<th>Table 2. Comparison of pMAPT, S100B, NEFL and GFAP levels between Lipid proteinosis and control groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lipid Proteinosis (n=15)</strong></td>
</tr>
<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td>pMAPT (pg/mL)</td>
</tr>
<tr>
<td>S100B (pg/mL)</td>
</tr>
<tr>
<td>NSE (ng/mL)</td>
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<tr>
<td>NEFL (pg/mL)</td>
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<tr>
<td>GFAP (pg/mL)</td>
</tr>
</tbody>
</table>

Note: S100B, NEFL, GFAP are expressed as mean ± SD, p-value obtained Student’s t-test. pMAPT and NSE are expressed as median [IQR], p-value obtained Mann-Whitney U test.

pMAPT: phosphorylated Microtubule Associated Protein Tau, S100B: S100 Calcium Binding Protein B, NSE: Neuron-Specific Enolase, NEFL: Neurofila ment Light Chain, and GFAP: Glial Fibrillary Acidic Protein.

The comprehensive analysis of neurodegenerative biomarkers, comparing patients diagnosed with LP to those in the control group, is detailed in Table 2. The biomarkers pMAPT, S100B, and GFAP showed higher levels in the LP group compared to the control group, but the differences were not statistically significant for pMAPT (p=0.331) and S100B (p=0.367). However, statistically significant differences were observed in the levels of NSE (p=0.028) and NEFL (p=0.003), with the LP group showing elevated levels. GFAP levels were also significantly higher in the LP group (p=0.002). These findings suggest potential biomarkers that may be relevant in the pathophysiology of lipid proteinosis.
### Table 3. ROC analysis results of NSE, NEFL and GFAP

<table>
<thead>
<tr>
<th>Variables</th>
<th>AUC</th>
<th>Cut-off level</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>p Values</th>
<th>95% CI for AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSE</td>
<td>0.718</td>
<td>3.59</td>
<td>60</td>
<td>87</td>
<td>0.042</td>
<td>0.526 - 0.909</td>
</tr>
<tr>
<td>NEFL</td>
<td>0.764</td>
<td>48.09</td>
<td>97</td>
<td>60</td>
<td>0.014</td>
<td>0.591 - 0.938</td>
</tr>
<tr>
<td>GFAP</td>
<td>0.813</td>
<td>233.67</td>
<td>93</td>
<td>93</td>
<td>0.003</td>
<td>0.658 - 0.968</td>
</tr>
</tbody>
</table>

AUC; area under the curve, NSE; Neuron-Specific Enolase, NEFL; Neurofilament Light Chain, and GFAP; Glial Fibrillary Acidic Protein.

### Figure 1. ROC curves of NSE, NEFL and GFAP

The predictive accuracy of NSE, NEFL, and GFAP in Lipoid Proteinosis was scrutinized using binary logistic regression and ROC analysis. The ROC analysis outcomes, depicted in Table 3 and Figure 1, demonstrated statistically significant predictive values, with GFAP emerging as a superior predictor for LP, as evidenced by its highest AUC. Complementing this, binary logistic regression analysis (refer to Table 4) affirmed the association between increased GFAP levels and LP, solidifying GFAP’s role as a critical biomarker in LP diagnosis.

### Table 4. Binary logistic regression analysis of NSE, NEFL and GFAP

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>SE</th>
<th>Wald</th>
<th>p Values</th>
<th>Odds ratio</th>
<th>95% CI for odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSE</td>
<td>-1.944</td>
<td>2.168</td>
<td>0.804</td>
<td>0.370</td>
<td>0.143</td>
<td>0.002 - 10.035</td>
</tr>
<tr>
<td>NEFL</td>
<td>-0.124</td>
<td>0.077</td>
<td>2.589</td>
<td>0.108</td>
<td>0.884</td>
<td>0.760 - 1.027</td>
</tr>
<tr>
<td>GFAP</td>
<td>-0.032</td>
<td>0.014</td>
<td>5.070</td>
<td>0.024</td>
<td>0.969</td>
<td>0.943 - 0.996</td>
</tr>
</tbody>
</table>

N=30, Nagelkerke R²= 0.630, Model: χ² = 19.20, p < 0.001. NSE; Neuron-Specific Enolase, NEFL; Neurofilament Light Chain, and GFAP; Glial Fibrillary Acidic Protein.

### Discussion

This investigation into the neurobiological implications of LP reveals a nuanced landscape of its effects on critical neurodegenerative biomarkers: pMAPT, S100B, NSE, NEFL, and GFAP. Our exploration deepens understanding of LP’s pathology and establishes connections with broader neurodegenerative processes.

The ECM1 mutation plays a central role in the pathogenesis of lipid proteinosis. This mutation damages the structural integrity and functionality of the extracellular matrix [8]. In the literature, the extracutaneous manifestations of lipid proteinosis, such as epilepsy and neuropsychiatric disorders, have been linked to the inhibition of Matrix Metalloproteinase-9 (MMP-9) activity by ECM1, a protein highly expressed in the brain [22, 23]. Consequently, impairments in ECM1 activity have been associated with structural changes in neurons, disruptions in synaptic transmission, and remodeling of the extracellular matrix. Similarly, our study supports these findings, as significant increases in NSE, NEFL, and GFAP levels were observed in LP patients. Contrary to prevalent expectations in neurodegenerative...
disease research (13-16), we observed no significant deviations in pMAPT and S100B levels among LP patients compared to controls. This finding diverges from the common narrative wherein pMAPT is implicated in tauopathies due to its abnormal phosphorylation and the resultant microtubule destabilization (24). Similarly, the absence of a significant increase in S100B levels, despite its known role in astroglial activation and neuronal injury (25), suggests that LP might modulate neurodegenerative mechanisms distinct from those observed in conditions like Alzheimer’s disease.

Conversely, the significant elevations in NSE, NEFL, and GFAP levels among LP patients highlight a distinct neurodegenerative signature (17-19). The marked increase in NSE and NEFL, indicative of neuronal damage and cytoskeletal disturbances, aligns with existing literature emphasizing their importance in maintaining neural integrity (26). This suggests that LP may precipitate neuronal injury through mechanisms involving cytoskeletal alterations, paralleling patterns observed in broader neurodegenerative disease contexts.

Furthermore, the pronounced elevation of GFAP levels in LP patients and its high predictive value for the disease accentuate the role of astroglial activation in LP’s pathology (20,21). This finding is not only consistent with the established function of GFAP as a marker for astrocytic response but also with Abdelhak et al.’s demonstration of GFAP’s predictive utility in neurological outcomes (27). The significant relationship between GFAP levels and LP revealed through our binary logistic regression and ROC analysis suggests that GFAP may be a biomarker for diagnosing and understanding LP.

Conclusion
This study highlights the neurobiological impacts of Lipoid Proteinosis on essential neurodegenerative biomarkers, offering new insights into the disease’s neurodegenerative aspects. The elevated levels of NSE, NEFL, and GFAP in LP patients underscore the potential of these biomarkers in understanding LP’s pathophysiology and in forming diagnostic criteria. In addition, the pattern of neurological involvement is usually slow and progressive. The use of these parameters may be useful in monitoring the neurodegeneration process in these patients. These contributions enhance the narrative of LP within the context of neurodegenerative diseases and set the stage for future research endeavors aimed at deciphering the complexities of this rare condition.

Limitations
The principal limitation of this investigation is the small sample size, which restricts the generalizability of the findings and precludes a detailed analysis correlating the severity of Lipoid Proteinosis with neurodegenerative biomarker levels. Given the phenotypic and genotypic variability of the condition, along with its unpredictable clinical trajectory, these results should be interpreted with consideration of these confounding factors.

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Ethical Approval: The study protocol adhered to the ethical standards established by the Declaration of Helsinki and Good Clinical Practice guidelines, and received approval from the Clinical Research Ethics Committee of Harran University (Date: 25/12/2023; decision number: HRU/23.24.26). Informed consent was obtained from all participants or their legal guardians.

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Data acquisition: S.T., M.A.
Analysis and interpretation: S.T., H.C., M.A.
Writing manuscript: S.T.
Critical revision of manuscript: H.C., M.A.
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