Review Article

Diagnostic Role of Tau Proteins in Amyotrophic Lateral Sclerosis: A Systematic Review and Meta-Analysis

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Received 26 August 2022; Revised 21 October 2022; Accepted 25 February 2023; Published 13 March 2023

Academic Editor: Vincenzo La Bella

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Background. Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that currently lacks reliable diagnostic biomarkers. The meta-analysis is performed with an aim to evaluate the diagnostic potential of cerebrospinal fluid (CSF) total tau (t-tau), phosphorylated-tau (p-tau), and their ratio in ALS patients.

Methods. A comprehensive search for literature published between the 1st of January 2000 and the 15th of May 2022 was performed in databases PubMed, medRxiv, and Google Scholar. The retrieved articles were first screened by title and abstract, and later, full-text screening was performed based on the eligibility criteria. Data on p-tau and t-tau levels and their ratio in ALS patients and controls were extracted, and a meta-analysis was performed using random-effects models in Review Manager version 5.4.

Results. Data were analyzed from seven studies reporting p-tau and t-tau levels and their ratio among ALS patients and controls. The number of total study participants was 1,100. In ALS patients, the levels of p-tau didn’t differ significantly with controls (standardized mean difference (SMD): 0.14 (95% CI: -0.41 to 0.70); \(p = 0.61\)). In contrast, there were significantly elevated levels of t-tau and significantly lowered p-tau/t-tau ratio in ALS (SMD: 1.76 (95% CI: 0.53 to 2.98); \(p = 0.005\) and SMD: -3.09 (95% CI: -5.33 to -0.86); \(p = 0.007\), respectively). Conclusion. Our meta-analysis study supports the role of core CSF biomarkers of neurodegeneration: t-tau and p-tau/t-tau ratio as a diagnostic biomarker of amyotrophic lateral sclerosis. This study found that t-tau is elevated while p-tau/t-tau ratio is lowered in ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS), formerly known as Lou Gehrig’s disease, is a progressive neurodegenerative disease with both upper and lower motor neuron dysfunction and results in death within a few years from diagnosis [1]. Its pathogenesis is not well known. The diagnosis of ALS is done mostly based on clinical findings and nerve conduction studies. Much effort is being put forth in the search for a biomarker that could help in the early diagnosis, disease progression, prognosis, response to therapy, and differentiation of the subtypes of the disease. Various biomarkers have been reported as potential biomarkers for ALS such as neurofilament light chain (NFL), TAR DNA Binding Protein 43 (TDP-43), and tau proteins [2]. Here, we present the systematic review and meta-analysis emphasizing the role of phosphorylated tau (p-tau), total tau (t-tau) and their ratio in the diagnosis of ALS.

Tau proteins are neuronal microtubule-associated proteins that help in the stabilization of microtubules, and they undergo many posttranslational modifications [3] including
2. Methods

2.1. Search Strategy. A comprehensive search for literature published between the 1st of January 2000 and the 15th of May 2022 was performed in databases PubMed, medRxiv, and Google Scholar. The search terms used were "total tau", "phosphorylated tau", "tau", "tau protein", "p-tau 181", "p-tau", "t-tau", "amyotrophic lateral sclerosis", "ALS", and "neurodegeneration" which were connected using "AND" and "OR" Boolean operators. The complete search string is mentioned in Supplementary Materials.

2.2. Study Selection. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline was followed throughout the study selection process. The studies retrieved after the literature search were exported to Mendeley Reference Manager and screened for duplicates. Duplicates detected were removed both manually and automatically. Studies comparing p-tau and t-tau levels or their ratio in ALS and controls were included. The studies were first screened by title and abstract, later followed by full-text screening based on the eligibility criteria, by AB and SS. Any discrepancies and doubts during the study selection process were solved through discussions and consensus from author ST.

2.3. Eligibility Criteria. Studies were included if they fulfilled the following criteria:

1. The study design was exclusively case-control
2. Any one among p-tau, t-tau, and p-tau/t-tau ratio was measured in ALS patients and controls
3. p-tau and t-tau were measured in CSF

Studies that evaluated p-tau and t-tau in histological specimens, postmortem tissues, studies whose full text was not accessible in English, editorials, review articles, and case reports were excluded.

2.4. Data Extraction and Risk of Bias Assessment. The data from the selected studies were extracted by AB and SS on a data extraction sheet with prespecified data variables including (1) author and publication year, (2) study country, (3) study design, (4) sample size, (5) mean age, (6) gender, and (7) findings in detail. For the findings, we extracted data on the level of phosphorylated tau, total tau, or their ratio in patients with ALS and controls. The description of assays utilized in the biomarker estimation was also extracted. Since few studies reported the levels of p-tau and t-tau in terms of median and interquartile range (median (IQR)), these were all converted into mean and SD applying the method proposed by Hozo et al. [6] and Bland [7].

The risk of bias among the studies was assessed by authors AB and ST using a modified ROBINS-I tool [8] under domains (1) selection of comparison groups, (2) bias due to confounding, (3) ascertainment of exposure, (4) measurement of outcomes, (5) missing data, and (6) reporting of results. We considered the risk of bias of a study to be low if all domains possessed low risk, moderate if any one domain possessed moderate risk, serious if at least one domain possessed serious risk, and critical if one of the domains possessed critical risk of bias.

2.5. Statistical Analysis. The extracted data were subjected to a meta-analysis which was performed by two authors (AB and SB) using Review Manager version 5.4 [9]. The heterogeneity among the studies was assessed using $I^2$ statistics. We considered the value of $I^2 < 25\%$, 25%-50%, and >50% as low, moderate, and high heterogeneity, respectively [10]. Likewise, a fixed- or random-effects model was selected based on the heterogeneity as presented by $I^2$ statistic. The chi-squared test was used to verify the heterogeneity among the included studies. The interstudy variance "tau2" was estimated using the DerSimonian and Laird method. The pooled standardized mean difference (SMD) was used to express the effect sizes on the difference between p-tau and t-tau levels and their ratio in cases and controls. The SMD was calculated using the inverse-variance method and expressed in 95% confidence interval. Finally, forest plots were generated for interpretation purposes. The meta-analytic model was adjusted for multiple hypothesis testing, and the significance level was established as $p = 0.05/3 = 0.016$ (no. of hypothesis tested = 3). Thus, $p \leq 0.016$ was considered a statistically significant finding. Publication bias was assessed by the visual examination of funnel plots consisting of standardized mean difference on the horizontal axis and standard error on the vertical axis. Likewise, to check the influence exclusion of larger studies on the pooled effect, sensitivity analysis was performed by removing one study at a time during the analysis.

3. Results

3.1. Search Results and Study Selection. The literature search retrieved a total of 11,304 studies. After screening by titles and abstract, 70 studies were subjected to full-text screening. Finally, 7 studies were included after the full-text screening. The detailed study selection process is displayed in the PRISMA flowchart in Figure 1.

The result of risk of bias assessment in each study is displayed in Supplemental Table 1. There were no studies reporting serious and critical risks of bias. Since all the included studies did not possess a low overall risk of bias, the risk of bias in our study is moderate. Figure 2 summarizes the risk of bias among the included studies.

3.3. Descriptive Characteristics of the Included Studies.
Table 1 shows the descriptive characteristics of the included studies. Seven case-control studies with a total number of 1,100 participants were included. Interestingly, all studies were from Europe where four studies [11–13, 16] were from Italy, and one each was from Greece [17], France [15], and Germany [14]. Among the participants, 634 were ALS patients and 466 were controls. The mean age of the participants was 53.11 years. In all studies, males were greater than females.

3.4. Biomarker Estimation Assay.
Except for Agnello et al. [12], who utilized the Chemiluminescence Immunoassay (CLIA) for the detection of tau proteins, all other studies utilized the enzyme-linked immunosorbent assay (ELISA). Lanzanster et al. [15] did not report on the sample handling and test validity. Among ELISA utilizers, the Innotest immune assay kit was used except for Paladino et al. [16] which used the Biosource immunoassay kit. The sample storage in all reporting studies was -80°C. Three studies [11, 13, 16] reported the variability coefficient of the test assay. Paladino et al. [16] also reported a detection limit of 12 pg/mL using Innotest ELISA (Table 2).

4. Meta-analysis
4.1. Tau Proteins in ALS versus Controls
4.1.1. p-tau Levels in ALS versus Controls.
Six studies were included in the meta-analysis on p-tau levels in ALS and controls, with a total number of participants being 577 (cases) and 356 (controls). Since the heterogeneity among the studies was high ($I^2$: 93%), a random-effects model was used to calculate the pooled standard mean difference (SMD). The forest plot (Figure 3) revealed an insignificant decrease of p-tau levels in ALS patients compared to controls (SMD: 0.14 (95% CI: -0.41 to 0.70); $p = 0.61$). The statistics provide evidence that p-tau levels in ALS patients do not differ significantly as compared to controls. The sensitivity analysis showed no fluctuation from the insignificant findings when larger studies were removed during the analysis. The funnel plot was symmetrical but contained one outlier study which is displayed in Figure 4.
4.1.2. t-tau Levels in ALS versus Controls. Seven studies were included in the meta-analysis on t-tau levels in ALS and controls, with a total number of participants being 634 (cases) and 466 (controls). The heterogeneity among the studies was high ($I^2$: 98%); therefore, a random-effects model was used to calculate the pooled standard mean difference (SMD). The forest plot (Figure 5) presented a significantly high mean concentration of t-tau levels in ALS patients compared to controls (SMD: 1.76 (95% CI: 0.53 to 2.98); $p = 0.005$). Thus, according to the statistics, patients with ALS have significantly higher t-tau levels. Sensitivity analysis however revealed fluctuation of the significant result to insignificant when larger studies except Lanznaster et al. [15] were removed during the analysis. The studies were close to the line of true effect with one outlier study as shown by the funnel plot in Figure 6.

4.2. p-tau/t-tau Ratio between ALS and Controls. Five studies were included in the meta-analysis on the p-tau/t-tau ratio in ALS and controls, with a total number of participants being 545 (cases) and 339 (controls). The heterogeneity among the studies was high ($I^2$: 92%); thus, a random-effects model was used to calculate the pooled standard mean difference (SMD). The forest plot (Figure 7) presented significantly low p-tau/t-tau levels in ALS patients compared to controls (SMD: -3.09 (95% CI: -5.33 to -0.86); $p = 0.007$). Thus, according to the statistics, patients with ALS have a significantly low p-tau/t-tau ratio. The sensitivity analyses showed no deviation from the significant findings when larger studies were removed during the analysis. The funnel plot is shown in Figure 8.

5. Discussion

Tau is a protein that aids in stabilizing the internal skeleton of neurons in the brain, which is required for nutrients and other essential substances to reach different parts of the neuron. Tau proteins are usually enriched with axons of central neurons and function to stabilize and maintain the stability of microtubules. Tau protein mostly undergoes posttranslational modification, which results in phosphorylation, the most important step for its interaction with microtubules. The alteration in microtubule-associated tau protein (tau metabolism), most importantly phosphorylated tau protein, is regarded as a prominent biomarker in different studies performed to access the levels of tau protein in CSF.

Mostly, it is thought that phosphorylation of tau protein is altered as tau is transported through the cell, but there is growing evidence that supports a theory that new tau protein is synthesized and is being phosphorylated immediately, making the level of p-tau in CSF nonsignificant [18, 19]. Studies on CSF total tau protein, phosphorylated tau protein, and the ratio of p-tau/t-tau protein are in limited numbers, and there are only a few studies describing all these parameters, in ALS. Increased levels of tau protein in CSF of ALS patients have been widely documented and suggested as potential biomarkers for ALS, although for the former these (p-tau and t-tau) have in general been represented as total tau. Meanwhile, some studies have failed to find similar increase in either CSF total tau or p-tau levels in ALS patients. Thus, it remains unclear if CSF tau can be used as a diagnostic marker for concomitant tauopathy in ALS.

This meta-analysis is aimed at studying the hypothesis that total tau (t-tau) and phosphorylated tau (p-tau) levels in CSF are diagnostic biomarkers for ALS. We compared the p-tau and t-tau levels as well as p-tau/t-tau ratio between patients with ALS and controls. The findings from the meta-analysis were contrary to our hypothesis of significant elevation of p-tau in ALS. We compared the standardized mean difference of p-tau and t-tau levels between ALS and controls. Our findings present an insignificant difference between the levels of p-tau among ALS and controls, and instead, we found that t-tau was significantly elevated and p-tau/t-tau ratio was lowered significantly in ALS patients as compared to controls. In the sensitivity analysis of t-tau in our study, we detected significant fluctuations of the pooled effect size, and therefore, we highly suggest more studies to be performed, investigating t-tau levels in ALS patients before the application of our findings in clinical practice. Undoubtedly, ALS patients do have a lower p-tau/t-tau ratio, and no significant fluctuations were detected in our sensitivity analysis.

The findings of our study are consistent with that of Scarafino et al. [13] who suggested the diagnostic role of t-tau in ALS. A number of studies have reported conflicting results to ours [16, 20, 21]. The low p-tau/t-tau ratio found in most studies has been highly correlated with the magnetic resonance imaging (MRI) findings and showed whole-brain grey matter atrophy and widespread loss of white matter integrity [22]. In a prospective study conducted by Xu et al. [23], both p-tau and t-tau had similar differences between ALS patients...
Table 1: Descriptive characteristics of the included studies.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Author and year of publication</th>
<th>Study country</th>
<th>Study design</th>
<th>Sample size (cases, controls)</th>
<th>Age (in cases)</th>
<th>Age (in controls)</th>
<th>Gender (M + F) (in cases)</th>
<th>Gender (M + F) (in controls)</th>
<th>Variable(s) measured</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Abu-Rumeileh et al., [11]</td>
<td>Italy</td>
<td>Case-control</td>
<td>80, 43</td>
<td>62.21 ± 12.41</td>
<td>62.37 ± 11.93</td>
<td>1.35</td>
<td>1.04</td>
<td>t-tau, p-tau/t-tau</td>
<td>ALS presented significantly high t-tau (p &lt; 0.001) and lowered p-tau/t-tau ratio (p &lt; 0.001) than in controls. No differences in the level of p-tau (p = 0.675).</td>
</tr>
<tr>
<td>2.</td>
<td>Agnello et al., [12]</td>
<td>Italy</td>
<td>Case-control</td>
<td>197, 35</td>
<td>65 (61-70)</td>
<td>67 (54-74)</td>
<td>1.21</td>
<td>1.21</td>
<td>t-tau, p-tau, p-tau/t-tau</td>
<td>ALS patients presented a significantly lower p-tau/t-tau (p &lt; 0.001) and significantly increased t-tau (p &lt; 0.001). No significant differences in the level of p-tau were observed (p = 0.172).</td>
</tr>
<tr>
<td>3.</td>
<td>Bourbouli et al., [17]</td>
<td>Greece</td>
<td>Case-control</td>
<td>32, 17</td>
<td>61.4 ± 9.47</td>
<td>59.7 ± 10.2</td>
<td>1.13</td>
<td>1.125</td>
<td>t-tau, p-tau, p-tau/t-tau</td>
<td>Significantly increased t-tau (p = 0.0055) in ALS as compared to controls. No significant differences in the level of p-tau (p &gt; 0.05). No significant differences in p-tau (p = 0.78), t-tau (p = 0.18), and p-tau/t-tau ratio (p = 0.48) in ALS and controls.</td>
</tr>
<tr>
<td>4.</td>
<td>Lanzmaster et al., [15]</td>
<td>France</td>
<td>Case-control</td>
<td>123, 90</td>
<td>66.06</td>
<td>67.45</td>
<td>1.66</td>
<td>1.32</td>
<td>t-tau, p-tau, p-tau/t-tau</td>
<td>No significant differences in t-tau levels between ALS and controls (p = 0.517).</td>
</tr>
<tr>
<td>5.</td>
<td>Paladino et al., [16]</td>
<td>Italy</td>
<td>Case-control</td>
<td>57, 110</td>
<td>60.33 ± 10.5</td>
<td>61.20 ± 10.7</td>
<td>1.71</td>
<td>1.75</td>
<td>t-tau</td>
<td>Significantly higher t-tau in ALS as compared to controls (p &lt; 0.001). p-tau/t-tau was significantly reduced (p &lt; 0.001). No significant difference in p-tau (p &gt; 0.05).</td>
</tr>
<tr>
<td>6.</td>
<td>Scarafino et al., [13]</td>
<td>Italy</td>
<td>Case-control</td>
<td>85, 51</td>
<td>57.96 ± 9.74</td>
<td>55.94 ± 9.74</td>
<td>1.78</td>
<td>0.75</td>
<td>t-tau, p-tau, p-tau/t-tau</td>
<td>Levels of p-tau did not differ significantly between cases and controls (p = 0.287). Significantly higher t-tau (p &lt; 0.001) while significantly lower p-tau/t-tau (p &lt; 0.001) in ALS as compared to controls.</td>
</tr>
<tr>
<td>7.</td>
<td>Wilke et al., [14]</td>
<td>Germany</td>
<td>Case-control</td>
<td>60, 120</td>
<td>67.34 (60.05-74.43)</td>
<td>64.14 (55.52-72.04)</td>
<td>1.30</td>
<td>1.07</td>
<td>t-tau, p-tau, p-tau/t-tau</td>
<td></td>
</tr>
</tbody>
</table>

Age is expressed in median (IQR) or mean ± SD or mean. Gender is expressed as the quotient of male and female.
and the control group. The study further found that neurofilament light chain levels were significantly higher in motor neuron diseases (MNDs) including ALS.

In another meta-analysis conducted by Noble et al. [24], both light and heavy chain neurofilaments were significantly increased in ALS as compared to controls. A study by [11] reported that neurofilament light chains (NFLs) were the best diagnostic measure of ALS and yielded the highest diagnostic performance with a sensitivity greater than 90%. However, both NFLs and tau levels are shown to increase

### Table 2: Biomarker estimation assay utilized in the included studies.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Study</th>
<th>Specimen</th>
<th>Assay</th>
<th>Sample storage</th>
<th>Test validity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>Agnello et al. [12]</td>
<td>CSF</td>
<td>CLIA (Lumipulse, Belgium)</td>
<td>-80°C</td>
<td>NR</td>
</tr>
<tr>
<td>3.</td>
<td>Bourbouli et al. [17]</td>
<td>CSF</td>
<td>ELISA (Innotest, Belgium)</td>
<td>-80°C</td>
<td>NR</td>
</tr>
<tr>
<td>4.</td>
<td>Lanznaster et al. [15]</td>
<td>CSF</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>5.</td>
<td>Paladino et al. [16]</td>
<td>CSF</td>
<td>ELISA (Biosource immunoassay kit, Belgium)</td>
<td>-80°C</td>
<td>Intra-assay CV: &lt;6%, detection limit: &lt;12 pg/mL</td>
</tr>
<tr>
<td>6.</td>
<td>Scarafino et al. [13]</td>
<td>CSF</td>
<td>ELISA (Innotest, Belgium)</td>
<td>-80°C</td>
<td>Interassay CV: &lt;10%</td>
</tr>
<tr>
<td>7.</td>
<td>Wilke et al. [14]</td>
<td>CSF</td>
<td>ELISA (Innotest, Belgium)</td>
<td>-80°C</td>
<td>NR</td>
</tr>
</tbody>
</table>

CSF: cerebrospinal fluid; ELISA: enzyme-linked immunosorbent assay; CV: coefficient of variation; NR: not reported.

![Forest plot](image-url)  
**Figure 3:** Forest plot presenting the standardized mean difference in the level of p-tau between patients with ALS and controls. p-tau is expressed in terms of pg/mL.

![Funnel plot](image-url)  
**Figure 4:** Funnel plot of studies analyzing p-tau in ALS and controls.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>ALS Mean</th>
<th>SD</th>
<th>Total</th>
<th>Controls Mean</th>
<th>SD</th>
<th>Total</th>
<th>Weight</th>
<th>Std. Mean Difference</th>
<th>Std. Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abu-Rumeileh et al. 2020</td>
<td>39</td>
<td>3.5</td>
<td>80</td>
<td>39</td>
<td>4.25</td>
<td>43</td>
<td>16.8%</td>
<td>0.00</td>
<td>[-0.37, 0.37]</td>
</tr>
<tr>
<td>Agnello et al. 2021</td>
<td>27.3</td>
<td>2.45</td>
<td>197</td>
<td>23</td>
<td>3.5</td>
<td>35</td>
<td>16.7%</td>
<td>1.63</td>
<td>[1.24, 2.02]</td>
</tr>
<tr>
<td>Bourbouli et al. 2017</td>
<td>41.5</td>
<td>15.5</td>
<td>123</td>
<td>49.8</td>
<td>27.9</td>
<td>90</td>
<td>17.4%</td>
<td>-0.24</td>
<td>[-0.52, 0.03]</td>
</tr>
<tr>
<td>Lanznaster et al. 2020</td>
<td>50.2</td>
<td>12.5</td>
<td>17</td>
<td>50.2</td>
<td>9.1</td>
<td>17</td>
<td>9.8%</td>
<td>0.07</td>
<td>[-0.28, 0.42]</td>
</tr>
<tr>
<td>Scarafino et al. 2018</td>
<td>35.08</td>
<td>13.58</td>
<td>85</td>
<td>34.06</td>
<td>15.98</td>
<td>51</td>
<td>16.9%</td>
<td>0.07</td>
<td>[-0.28, 0.42]</td>
</tr>
<tr>
<td>Wilke et al. 2014</td>
<td>40.5</td>
<td>5.68</td>
<td>60</td>
<td>41.5</td>
<td>3.6</td>
<td>120</td>
<td>17.2%</td>
<td>-0.23</td>
<td>[-0.34, 0.08]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>356</td>
<td>100.0%</td>
<td>0.14</td>
<td>[-0.41, 0.70]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: $I^2 = 0.45, X^2 = 72.47, df = 5 (P < 0.00001); I^2 = 93$

Test for overall effect: $Z = 0.50 (P = 0.61)$
proportionally to the extent of neurodegeneration and were not specific to ALS. Likewise, different studies have suggested that these CSF biomarkers have a diagnostic significance [13, 25]. Further, Jeganathan et al. [26] proposed that the distinction between ALS subtypes can be accomplished based on these CSF biomarkers with NFLs being superior to tau proteins in the distinction.

Data were analyzed from 7 studies reporting p-tau and t-tau levels among ALS patients. The p-tau value was found significant for Abu-Rumeileh et al. [11] and Agnello et al.
Except for two studies [15, 16], the t-tau levels were found to be significantly higher in ALS in all the studies. Apart from the study of Lanznaster et al. [15], all other studies reported positive values of SDM and corresponding 95% CIs. The results of this meta-analysis are in accordance with the role of total tau protein in the pathogenesis of amyotrophic lateral sclerosis and support it as one of the diagnostic biomarkers for ALS. The most important finding of this meta-analysis is the insignificance of determining CSF p-tau protein in ALS patients which emphasizes the need to do more studies in the specific involvement of p-tau protein in the pathophysiology of ALS.

The possible mechanism behind altered tau protein metabolism can be attributed to the increased posttranslational modifications of tau proteins in neuronal damage. Tau proteins undergo extensive phosphorylation when in contact with the microtubules through the microtubule-binding region (MTBR). Thus, modified tau is seen as pathological tau deposition on the neuronal tissue [27–29]. Elevated phosphorylated tau in CSF is thus an indication of ongoing neuronal pathology. Soma-localized tau exhibits a higher degree of phosphorylation, and an isolated elevation of p-tau can possibly be present in soma-localized neuronal degeneration [30]. Further, the phosphorylated tau dissociates producing soluble tau isoform which freely polymerizes and leads to fibril formation, and this probably explains why ALS patients presented with a significantly lower p-tau/t-tau ratio in CSF. Considering this fact, the levels of p-tau in ALS from our findings were questionably insignificant, and this is because the ratio indicates the extent of phosphorylation with respect to total tau, which is low due to dissociation of p-tau into fibrils. In contrast, p-tau alone in ALS could differ undoubtedly and moreover depends upon the disease duration and subtype of ALS present [16]. The polymer of such fibrils deposit as neurofilaments and is a dissociation product of phosphorylated tau, and this also explains the significant elevation of NFLs as compared to p-tau and t-tau in different studies [31].

The strength of our study is that it is the first meta-analysis conducted which investigated the significance of p-tau protein in the diagnosis of ALS. Most of the meta-analyses to date have compared these parameters in Alzheimer’s disease, and a very small number of analyses have been performed with emphasis on ALS [32]. The diagnostic insignificance of p-tau in ALS is the novel finding of this meta-analysis. Also, the included studies utilized the same immunoassays except by Agnello et al. [12] which ensured equal sensitivity of the test assay. Our study, however, has some limitations. First of all, there was substantial heterogeneity among the included studies which might possibly be due to the difference in sample sizes and control group selection in each study. While few studies assigned healthy individuals as controls, few assigned that to individuals with other neurodegenerative diseases. Secondly, the levels of these molecules in different subtypes and variants of ALS could not be accessed. Likewise, the statistics of the publication bias including Egger’s test could not be generated. And finally, insufficiency might have arisen due to the potential omission of valuable data.

6. Conclusion

Our study was designed to test the hypothesis regarding tau proteins as a diagnostic biomarker of amyotrophic lateral sclerosis. Our meta-analysis found that t-tau and p-tau/t-tau ratio can be used as a diagnostic marker in amyotrophic lateral sclerosis since the former is elevated and the latter is lowered significantly. Elevated total tau and a lower phosphorylated tau/total tau ratio in CSF showed significant association suggesting the role of the two as a potential diagnostic biomarker for this neurodegenerative disease. However, further studies are needed to corroborate these
findings to allow the application of estimating tau proteins while dealing with ALS in clinical practice.

**Data Availability**
All the required information is in the manuscript itself.

**Conflicts of Interest**
The authors have no conflict of interest to declare.

**Authors’ Contributions**
AB, ST, and SS wrote the entire manuscript. SW, SB, OM, and SKM reviewed and edited the manuscript.

**Supplementary Materials**
Search string: (“phosphorylated tau” OR “p-tau” OR “p-tau 181” OR “total tau” OR tau OR “t-tau” OR “tau protein”) AND (“amyotrophic lateral sclerosis” OR ALS OR neurodegeneration). (Supplementary Materials)

**References**


