Mutation in microtubule-associated protein tau MAPT coding gene and its correlation with Alzheimer’s disease

Sabah Subhi Ismael*, Sarab D. Al-Shamaa
Department of Biology, College of Science, University of Mosul, Mosul, Iraq

Article History:
Received on: 14 May 2020
Revised on: 01 Jun 2020
Accepted on: 29 Jun 2020

Keywords:
Alzheimer disease, MAPT gene, PCR technique, tau protein

ABSTRACT
Alzheimer’s disease (AD) is a progressive irreversible neuronal dysfunction characterized by progressive memory loss along with neuropsychiatric symptoms and a decline in daily activities. Disturbances in microtubule-associated protein tau (MAPT) gene expression result in disruption of the neuronal cytoskeleton and formation of neurofibrillary tangles. The study aims are to highlight the correlation between MAPT gene’s exons mutations and AD. Beside the possibility of utilizing serum’s tau protein concentration as an indicator for AD due to the accumulation of intracellular neurofibrillary filaments of highly phosphorylated Tau in AD patient’s brain. DNA had been extracted from participant’s blood that divided into three groups 30 participants in each ,AD patients ,Positive family history and healthy or control groups. The results of this research showed that serum’s Tau protein concentration in AD patient group was significantly higher than healthy control and positive family history group and according to this result serum’s tau concentration could be utilized as a good indicator for AD. Beside mutation in each 1,9 and 13 exons had been identified by PCR product analysis utilizing specific primers for each , Amplification PCR products in exon (1) showed 428bp band in AD patients group does not exist in 26.66% and in positive family history for AD group does not exist in 23.33%, In exon (9) PCR product of 604bp band in AD patients group does not exist in 53.33%, and in positive family history group does not exist in 43.33%. While amplification PCR product of exon 13 showed 299bps band in all healthy control and positive family history but not in AD patient group instead it band in 263bp had been appeared in 36.66%. These results confirmed the important role of tau protein and its coding gene in the pathology of AD.

INTRODUCTION
Alzheimer’s disease (AD) is an irreversible, progressive neurodegenerative disease that gradually devastates memory and thinking skills, it was the most common cause of dementia .The most important AD hallmarks were the deposition of two proteins: one of them accumulates intracellularly called tau protein and the other accumulates extracellularly called β-amyloid (Selkoe and Hardy, 2016; Sánchez-Juan et al., 2019; Braak and Braak, 1991). Tau Tubulin-Associated Unit is a multifunctional low molecular weight protein, found in the normal cells nucleus, as a dephosphorylated protected from interact with the genome , but modifiable Phosphorylated tau
may change its function thus enhance genome vulnerability and neurodegeneration as in AD (Maina et al., 2016). Tau protein support and stabilize microtubules of neuronal cells in the central nervous system, and many researchers proved its essential role for normal synaptic mechanisms, also for maintenance of axonal transport and providing linkage for signal transduction (Ittner et al., 2010; Martin et al., 2011). It may be unregulated in AD specially through interaction with genetic risk factors in β-amyloid dependent or β-amyloid independent manner (Dourlen et al., 2019). Hyper-phosphorylated tau protein lead to the formation of neurofibrillary tangles, which are neurodegenerative disorders cause (Hallinan et al., 2019). While Extracellular accumulations of β-amyloid form senile plaques, the number and size of these plaques were partially restored when human tau was reduced (DeVos et al., 2018). Many researches revealed that tau protein was more effective than β-amyloid to strongly cause memory loss and neurodegeneration disorders (Selkoe and Hardy, 2016; Franzmeier et al., 2019; Gordon et al., 2019). Thus Tau is used for detecting early cognitive alters in preclinical AD (Ossenkoppele et al., 2019). The genetic architecture of Alzheimer’s disease (AD) is not completely understood (Zhang et al., 2019). There are more than 45 genes or loci had been linked to the peril of AD progress (Dourlen et al., 2019), but most Genetic studies had been focused on the β-amyloid and tau proteins coding genes (Kunkle et al., 2019). Tau is a product of the Microtubule Associated Protein (MAPT) gene, located on chromosome 17q21 (Andreadis, 2005). This gene has 16 exons, of which, exon 2, 3, 4A, 6, 8, 10 and 14 are alternatively spliced (Andreadis, 2005). When MAPT expression levels increase the severity of neurodegeneration in Alzheimer’s disease also increase (Grothe et al., 2018). Recent studies detected that Over-expression of mutated human tau (P301L) in mouse results in neuronal silencing and loss of excitatory neurons in entorhinal cortex, leading to destroy memory and tau propagation (Fu et al., 2019; Busche et al., 2019; Fu et al., 2017) Persons of Positive family histories of AD have alleles that more risk than healthy ones to be affected (Zhang et al., 2019). The level of tau have been strongly associated with neuronal death and dysregulation as in AD plasma total tau is elevated, (Mielke et al., 2018) and also increased in cerebrospinal fluid when compared to normal persons at the same age (Pillai et al., 2019). For this reason the aim of this research focused on the level of serum’s tau proteins and the mutation of MAPT gene that express this protein as an indicator for Alzheimer early detection.

MATERIALS AND METHODS

Blood Samples collection

Participant’s blood samples had been collected from August 2019 till January 2020, from Duhok Governorate divided into three groups, AD patient’s group that showing diagnostic criteria of Alzheimer disease, positive family history’s for AD group that had no Alzheimer’s criteria but their parents or one of them had, healthy control group that hadn’t or their parents Alzheimer’s criteria. 5ml from vein puncture blood had been divided into 2ml put in EDTA tube. And 3 ml was put in gel tube to be centrifuged after 2 hours for clotting and got serum; both samples were kept in (~-20 °C) until using.

Concentration of tau protein

Tau protein concentration had been measured by Enzyme-Linked Immunosorbent Assay (ELISA) kit that produced by (Bioassay technology Laboratory Company).

DNA extraction

DNA from whole blood was extracted by using Genaid DNA extraction kit.

DNA concentration & purity

Extracted DNA concentration ng/μL were estimated by Nano drop machine, at the same time the purity of the extracted DNA had been estimated by the ratio of optical density 260/280 to detect the presence of RNA and protein

Agarose gel electrophoresis: agarose gels concentrations 2% had been make ready by adding Agarose powder (1 gm.) to the 50 ml of 1X TBE buffer.

DNA loading and Electrophoresis

DNA Quality and integrity detected by electrophoresis on agarose gel concentration 1% by mixing 5 μL of extracted DNA with 2 μL of loading dye in 0.2 ml tube, the result as shown in Figure 1.

Primer selection

For amplification of MAPT gene the primers mentioned by (Rizzu et al., 1999) had been utilized, as shown in Table 1.

The PCR optimizing reactions were accomplished according to rizzu etal 1999

PCR components

For PCR products analysis electrophoresis was done in a gel concentration 2% by mixing 20 μL of the PCR products and loading them directly to the gel, with the use of 50 bp DNA ladder as a size marker.
to detect the product size then the gel connected to electrical field in the power 100 volt (6.666 volt/cm) for 40 min. then the gel pictured when using UV trans illuminator and documented by camera.

The PCR program used for the amplification is represented in Table 2 for Exon 1, Exon 9 and Exon 13.

**Analysis of PCR product**

PCR products of the MAPT gene was analyzed by electrophoresis on 2 % agarose gel with the use of 50bp DNA ladder as a size marker to check the molecular weight of the amplifications. The electrophoresis was using 5 μL of the PCR product subjected to electric field power 100 volt (6.666 volt/cm) for 40 min then the gels were visualized using UV trans-luminator and documented by camera.

**RESULTS AND DISCUSSION**

**Tau protein concentration**

The results of this research revealed significantly increase in serum’s tau protein concentration at P value <0.05 in AD patient group compared with positive family history and healthy control groups, the results were 1.09±0.18 ng/L, 0.84±0.17 ng/L, 0.62±0.08 ng/L respectively, as revealed in Figure 2. Serum tau was higher in AD group due to the over-activated of microglia, and loss of their phagocytosis abilities resulting in uncontrolled inflammation (Mrak and Griffin, 2005). This result demonstrate that serum tau protein are strongly associated with AD, and it could be used potentially for its early diagnosis, because there is no test till now for early diagnosis, thus it can be depended on the level of serum’s tau protein that had more advantages than the CSF or neuroimaging measures due to its feasibility at the population-level, low cost, and invasiveness.

The comparison of serum tau level between each two groups are found that AD patient has signi-
Figure 3: PCR Amplification product of MAPT gene exon 1

Figure 4: PCR Amplification product of MAPT gene exon 9

Table 1: PCR primers for Genomic Amplification of Tau Exons (5 → 3)

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward</th>
<th>Reverse</th>
<th>Exon size(b)</th>
<th>PCR product(bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CAACACTCTCAGAACTTATC</td>
<td>CAGTGATCTGGGCTGCTTG</td>
<td>150</td>
<td>228</td>
</tr>
<tr>
<td>9</td>
<td>CGAGTCTGGCTTTCACTCC</td>
<td>CTTCAGGTCAGCCACATACC</td>
<td>266</td>
<td>379</td>
</tr>
<tr>
<td>13</td>
<td>CTTTCTCTGGCACTCTCTC</td>
<td>CCTCTGCAATTATTGACCG</td>
<td>208</td>
<td>299</td>
</tr>
</tbody>
</table>
cantly higher serum tau than the Healthy control group at \( P < 0.05 \). Also positive family history for AD has significantly higher serum tau than the healthy control group.

the results of this research had been accepted with other researcher’s results such as (Mielke et al., 2018). which found elevation in plasma’s total tau protein in AD patients compared with healthy individuals, (Pillai et al., 2019) that also showed tau increase in AD cerebrospinal fluid compared with normal persons at the same age (Cummings, 2011) confirmed that CSF tau proteins were used as a biomarker in clinical test, (Shekhar et al., 2016) .In other study revealed that the concentration of serum’s Tau was significantly higher in AD when compared with control healthy ones. Beside the result of (Salama et al., 2018) proved that the Serum’s Tau was higher in patient with AD than the Parkinson disease. Increased expression of tau protein may lead to the risk of a pathological accumulation and deposition of the protein in its fibrillary form, as confirmed by (Götz et al., 2007)in their study on animal models. Another study accomplished by (Toral-Rios et al., 2020) demonstrated that the over expressed of tau protein lead to the deposition and accumulation of this protein as neurofibrillary tangles (NFTs) in the brain tissue of temporal lobe epilepsy TLE patients with an increase of glycogen synthase kinase-3 beta, GSK3b hyperactivity may cause posttranslational modifications of some microtubule-associated proteins (MAPs), specially tau.

**MAPT** coding gene mutation

Three exons of **MAPT** coding gene had been chosen in this research for mutation detect in participant’s DNA, Exon 1, Exon 9 and Exon 13. Amplification PCR products in exon (1) showed the existence of 428bp band in all healthy control but in AD patients group doesn’t exist in 26.66% and in positive family history for AD group doesn’t exist in 23.33% as shown in Figure 3. In exon 9 PCR product of 604bp band had been existed in all healthy control, but in AD patients...
group doesn’t exist in 53.33% and in positive family history for AD group doesn’t exist in 43.33% as shown in Figure 4.

While amplification PCR product of exon 13 showed 299bps band in all healthy control and positive family history but not in AD patient group instead another band in 263bp bands had been appeared in 36.66% of AD patient as shown in Figure 5.

Tau is a microtubule-associated protein, which is highly expressed in the central nervous system as well as ocular neurons and stabilizes microtubule structure. It is a phospho-protein being moderately phosphorylated under physiological conditions but its abnormal hyper-phosphorylation or some post-phosphorylation modifications would result in a pathogenic condition, microtubule dissociation, and aggregation. The aggregates can induce neuro-inflammation and trigger some pathogenic cascades, leading to neurodegeneration (Mishan et al., 2019). Tau mutation that occurred in different percentage have a central role in Alzheimer’s disease and other tauopathies as demonstrated by many researcher such as (Andreadis, 2005; Strang et al., 2019) which concluded that mutations in the tau coding gene may affect microtubule construction, resulting in increased tau self-aggregation.

Tau protein contains three domains the N-terminal region (“projection domain”), modulated by exons 2 and 3, interacts with the plasma membrane (Pooler and Hanger, 2010; Lebouvier et al., 2009; Lee, 2005). Tau protein binds microtubules through some repeated domains associated microtubules (R1–R4) (encoded by exons 9–12) located at the C-terminus of the molecule, Each repeat consists of a highly conserved 18 residues that are imperfectly repeated three times in the fetal tau protein and four times in the adult specific form. The repeats are separated from each other by 13- or 14-residue spacer regions (Brandt and Lee, 1993).

The microtubule-associated protein tau is integral to the pathogenesis of AD. Rare mutations in the MAPT gene cause familial dementia syndromes (Lee and Leurgens, 2012).

(Mukrasch et al., 2005) confirmed that Microtubule Binding Domain MBD coding by exons 9–12; amino acid 244–368; of MAPT coding gene was the essential region for the primary function of tau that is MT binding, polymerization and dynamics regulation. Most of missense mutations localized in MBD have been demonstrated to confer a reduced ability to interact with tubulin, slowing and decreasing MT formation. This “loss of function” can lead to cytoskeleton disruption, affecting physiological cell functions (Iqbal et al., 1986).

(Liu and Gong, 2008) in their research also revealed that several missense mutations in tau coding gene exons 1, 9, 11, 12 and 13 influenced MT binding or tau conformation.

CONCLUSIONS

MAPT protein had an important role in AD disease due to its role for microtubules assembling. Mutation that occurred in specific protein domain of MAPT coding gene which attached to the tubulin of neuronal axons had an important role for disrupting this assembling and neuronal pulse defects. MAPT coding gene exons 1,9 and 13 responsible for protein domains expression that attached neuronal axons and any mutation in these exons may affect these attachments, beside hyper-phosphorylation of tau protein may lead to the microtubules dissociation and aggregation which lead to different pathological effects.

Different Exons of MAPT gene mutation had been revealed in this research utilizing PCR technique such as band missing or bands appeared in different position in PCR product reflected the expressing protein. Serum Tau protein concentration differentiated between AD patient, positive family history for AD and healthy control. Hence, Tau protein in serum can be used as an indicator for AD.

ACKNOWLEDGEMENT

We would like to thank laboratory staph at veterinary/Duhok directorate, and each of Msc. Swaila Mekael, Msc. Khairi M. Qasim and both neurologist Dr. Hiwa Adulsalam and Dr. Dilshad Khursheed.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

REFERENCES


Martin, L., Latypova, X., Terro, F. 2011. Post-translational modifications of tau protein: Impli-


