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EVALUATION STUDY OF NEURODEGENERATIVE DISORDER IN RELATION TO THYROID HORMONES BY MEASURING NEUROFILMENT LIGHT PROTEIN (NFLP) IN TYPE II DIABETIC PATIENTS IN THI-QAR / IRAQ

Zainab Ali Kadhem[1], Hassan Tuhmaz Hamad [2]

[1]Asst. Prof. PhD. Biochemistry. Department of Biochemistry, College of Medicine, University of Thi-Qar. Iraq. Email: zainab-ali@utq.edu.iq BOI: https://orcid.org/0000-0001-6002-9700
[2]Asst. Prof. PhD. Biochemistry. Department of Biochemistry, College of Medicine, University of Thi-Qar. Iraq. Email: hassan-tz@utq.edu.iq

ABSTRACT
Background: Neuro light filament proteins are the smallest type of neurofilament proteins and the early indicator of neuronal cytoskeleton damage, its useful to diagnose neurodegenerative disease in relation to other metabolic complication associated with diabetic mellitus.
Aims: This study was aimed to comparative evaluation of the neurodegenerative status in type II diabetic patients with and without autoimmune disease by measuring nerofelment protein as an early neuro damage marker.
Methods: This study is a case control study, included 308 subject aged between 26-65 year, mean± SD (46.01 ±1.132), they were classified in three group 100 healthy individuals as control group (86.11 %) female and (13.89%)), the others were 208 patients ((83%) female and (17%) male)), diagnosed with type II diabetes mellitus with normal thyroid functions (T2DM) and others 108 type II DM with hyperthyroidism (T2DMHT).
Results: All patients associated with elevation in neurofilament protein and there were significant differences in NFLP level between the study groups, it is significantly elevated in T2DMHT patients as compared with DM and control group (159.8±21.90, 71.86 ±19.8, 0.0 ±0.0, on another hand there was a significant decrease in TSH (mIU/ml) level in serum of T2 DM HT (mean ± SD: 0.098 ±0.09), and significant increases in T4 (mean ±SD: 138.37±26.7) and T3 ($p \leq 0.001$) (3.23±0.06) as compared to control group and T2DM cases. Fasting blood sugar significantly increased in T2DMHT (398 ± 12.38) comparing with T2DM and control group,) NFLP positively related with F.B.S ($R^2 = 0.791$) and T3 ($R^2 = 0.933$) but weakly with T4 (0.458). and T3 positively related with FBS (R2:0.86), but T4 was weakly related with FBS (R2: 0.299
Conclusion: Type II DM patients with hyperthyroidism complicated with high level of NFLP and neurodegenerative disorder more than type II DM patients with normal thyroid function. The neurodegenerative status increased with thyrotoxicosis.

KEYWORDS: Type II Diabetes mellitus, Hyperthyroidism, Neurofilament light protein, Neurodegeneration.

INTRODUCTION:

Individual with diabetes especially type II can be develop autoimmune thyroid disease, the interaction between two conditions can lead to metabolic complication associated with neuropathy. Type II Diabetic mellitus (T2DM) is the high prevalence disease in the world wide and in Iraq [1]. Patients with T2DM can be associated with different complication as a result of hyper-glycemia and insulin resistance [2,3]. The abnormalities in thyroid gland (Hypothyroidism or Hyperthyroidism) are more common in T2DM, both thyroid hormones (THs) T4 and T3 are essential for the development and functionality of humane brain and nervous system, they influence neural and glial cell differentiation and migration, neurogenesis, synaptogenesis [4,5]. The defect in THs can leads to permanent or irreversible brain damage, defect in the neuronal growth and damage or reduce the size of axonal [6].
Actually, it is necessary to identify and qualitative the axonal damage in order to assessment and management neurological defect. Recently neurofilment proteins (Nfs) are the useful markers of neuroaxonal integrity or damage, those proteins classified as a type of intermediate filaments type IV [7]. Nfs proteins are heteropolymer consist of five type of subunit proteins are alpha internexin, peripherin, neurofilment light (NFL (70KD)), neurofilment medium (NFM (160 KD)), neurofilment heavy (NFH (200 KD)). Nfs are measuring about 10 nm in diameter and many micrometers in length. They form the neuronal cytoskeleton through interacted together with microtubules (25nm) and microfilaments (7nm) and expressed in large caliber myelinated axons [6,8]. During neuroaxonal damage Nfs level increased in cerebrospinal fluid (CSF) and blood, the releasing of these proteins is indicator, biochemical marker of neuronal damage, inflammation, traumatic, cerebrovascular and neurodegenerative disease. Especially NFL has

become promising early diagnostic biomarker in many neurodegenerative conditions because of it has low molecular weight comparing to other Nfs proteins. therefor the assessment of these neuro-proteins assist in the early diagnosis and therapeutic targets [9,10].

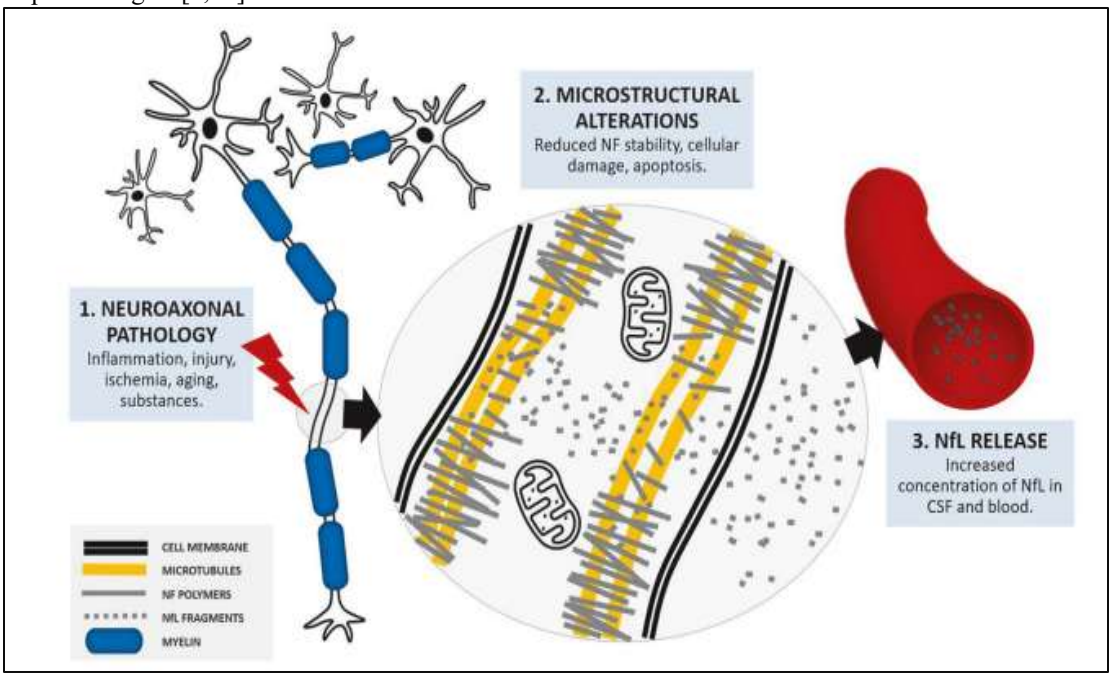

Figure 1: The neuroaxonal pathology, microstructural alterations and releasing of neurofilament light protein [10].

This study aimed to determine serum level of light neurofilament protein as a marker of neuroaxonal damage and neurodegeneration state and its relation to hyperglycemia and thyroid hormones (T4,T3) in type II diabetes mellitus patients with and without hyperthyroidism.

METHODS

This study is a case control study, about 308 subject were collected included 208 patients, aged between 26-65 year, mean± SD (46.01 ±1.130), their body mass index (BMI) was 23.11 ±1.25. they were received Diabetic and Endocrine Center at Thi-Qar province, diagnosed with type IIDM duration of disease (2.4 ± 0.53 year) and classified according to the thyroid function results in the two groups: 100 patients ((83%) female and (17%) male) diagnose with normal thyroid functions. The second group included 108 patients ((86.11 %) female and (13.89%) male) with thyroid dysfunctions, in this study excluded criteria patients with obese, smoking, hypertensive, diabetic nephropathy, and retinopathy. Control group included 100 healthy individuals, their age 26-65year mean± SD (44.5 ± 2.074) ((84.22%) female and (15.78%) male) and BMI was (22.57±0.44). Exclusion criteria of type II DM patients associated with abnormal BMI (≥ 25), cardiovascular disease, nephropathy or retinopathy.

=Biochemical analysis, of fasting venous blood samples (3ml) were collected then serum samples separated and storage according to the standard method [11]. Serum biochemical detection of fasting blood sugar (F.B.S.) using kit from Biomagreb. TSH, T4, and T3 were estimated by autoanalyzer cobas e411 by using kits from Roche Elecsys. NFLP (pg/ml) were detected by kit from Elabscience (E-EL-H2020) using ELIZA technique [12].

Statistical analysis:

Statistical package for social sciences (IBM SPSS) software version 23 was used for data analysis. Tests were used are chi-square, ANOVA, multiple comparison and post hoc test (Tukey test). Continuous variables expressed as mean and standard deviation (mean ± SD). P-values ≤ 0.05 was considered statistically significant and P value >0.05 was referred to the non-significant differences. Pearson test used to calculate the correlation between variables [13].

RESULTS

Table 1 and figure 2 show no significant different ($p > 0.05$) in the concentrations of TSH, T4, and T3 between control group (1.80 ± 0.51, 96.44 ± 16.06, and 1.16± 0.36) and T2DM group (1.74 ± 0.57, 101.01±21.7, and 1.15 ±0.33). but there was a significant increase ($p \leq 0.05$) in these parameters in T2DMHTwhen compared to control and T2DM with normal thyroid function groups (0.98 ±0.09, 138.37±26.7 and 3.23±0.06)

Table 1: Concentration of TSH (mIU/ml), T4(nmol/L), T3 (nmol/L) in 100 healthy subjects (control) and 208 patients with type II DM with normal and with abnormal thyroid functions.

Study group	no	TSH (mIU/ml)		T4(nmol/L)		T3(nmol/L)	
		mean ±SD	p- value*	mean ±SD	p-value*	mean ±SD	p-value*
Control	100	1.80± 0.51	0.675	96.44± 16.06	0.383	1.16± 0.36	0.99
T2DM	100	1.74±0.57	0.675	101.01±21.7	0.383	1.15 ±0.33	0.99
T2DMHT	108	0.098 ±0.09	0.001	138.37±26.7	0.001	3.23±0.06	0.001

*p-value > 0.05 : no significant differences, p- value ≤ 0.05 revered to significant different.

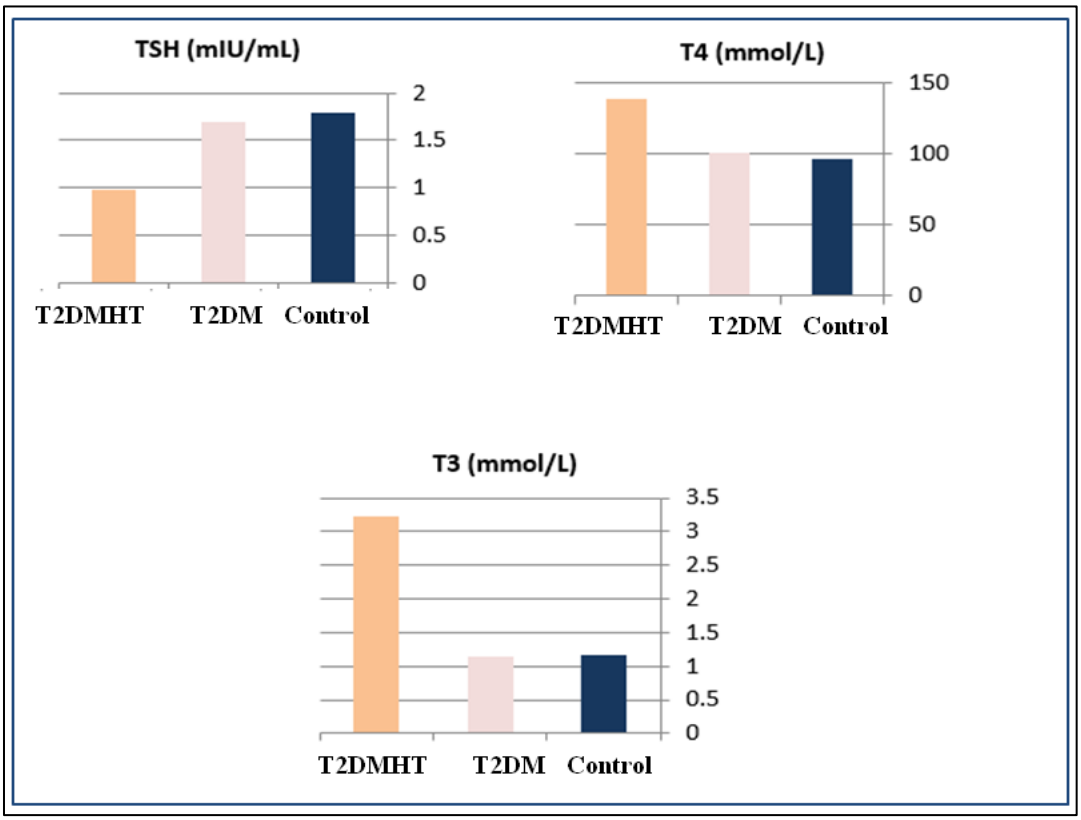

Figure (2): Serum concentration of TSH (mIU/ml) and thyroid hormones (T4 (nmol/L), T3 (nmoLL)) in type II DM with hyperthyroidism (T2DMHT), type II DM with normal; thyroid function (T2DM) and healthy subject (control)

In table 2 , there were a significant differences (p ≤ 0.05) in the concentration of FBS between all study subjects , FSB significantly elevated in type IIDMHT (398 ± 12.38) as compared with group of type IIDM with normal thyroid function (203 ±15.50) and control group (79.76 ± 7.43).

Table2: Concentration of FBS in control group (100) and patients group type II DM with normal thyroid function(100) and Type II DMHT (108).

Study groups	no	Fasting blood sugar mean ±SD	P value*
Control	100	79.76 ± 7.43	0.001
T2DM	100	203 ±15.50	0.001

T2DMHT	108	398 ± 12.38	0.001

*p-value ≤ 0.05 mean there was significant differences

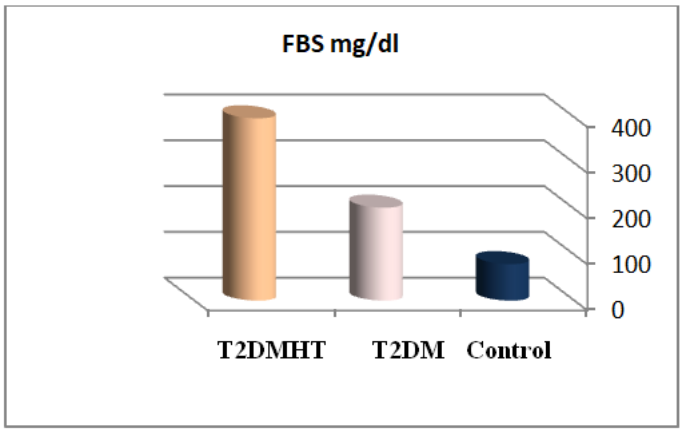

Figure 3: Serum concentration of FBS (mg/dl) in 208 patient and control group

In table 3, the concentration of LNFP significantly increased ($p \leq 0.05$) in diabetic mellitus with normal thyroid function (71.86 ± 19.8 ng/ml) as compared with control group (0.00 ng/ml) and also there were a significant increase ($p \leq 0.05$) in diabetic patients with thyroid dysfunction (159.8 ± 21.90 ng/ml) as compared with diabetic patients with normal thyroid function and control group (see figure 5).

Table 3. The concentration of LNFP (ng/ml) in patients with type II DM with normal thyroid function (T2DM) and patients with hyperthyroidism (T2DMHT).

Study groups	no	LNFP (ng/ml) (mean ±SD)	P- value*
Control	100	0.0 ±0.0	0.001
T2DM	100	71.86 ±19.8	0.001
T2DM HT	108	159.8±21.90	0.001

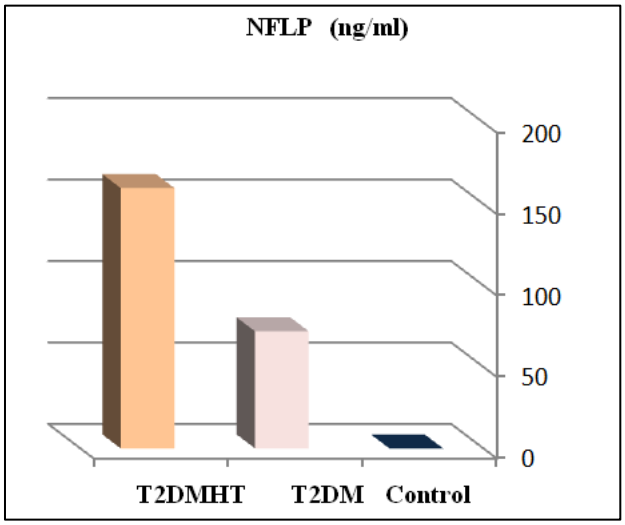

Figure 5: Serum concentration of NFLP (ng/ml)in all subject group.

Figures 6 shows the co relation between serum concentration of T4 and FBS in type IIDM with hyperthyroidism, there was a weak correlation ($R^2 = 0.299$) .

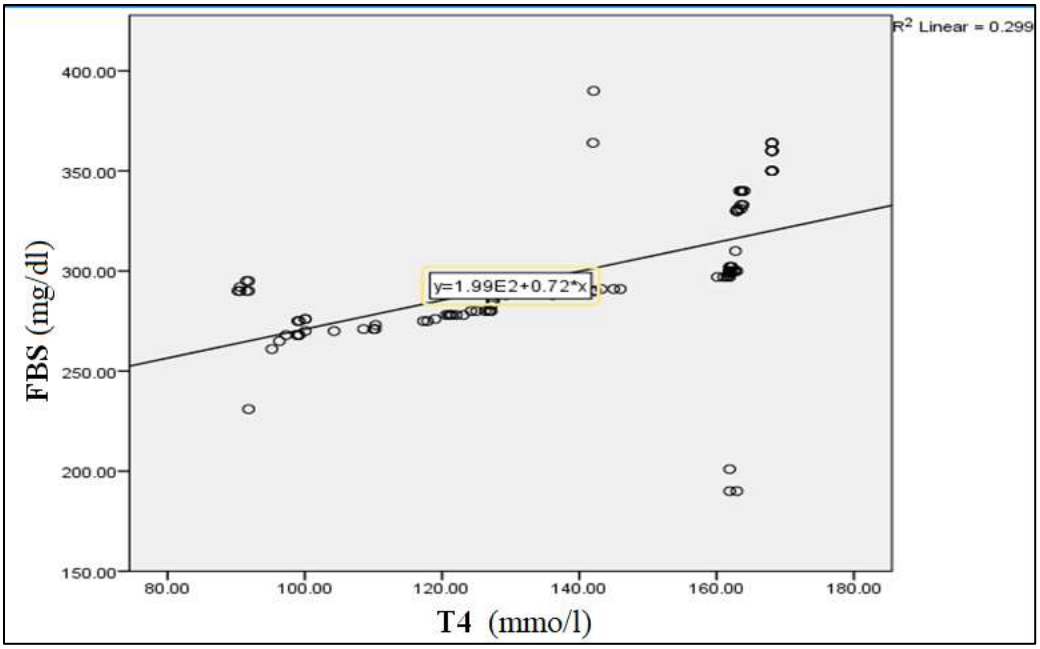

Figure 6: the correlation between serum T4 and FBS in 108 patients with type II DM

A strong correlation between (R^2= 0.864) the rising T3 and the increasing in FBS level in serum of hyperthyroidism case and DM patients as in figure 7.

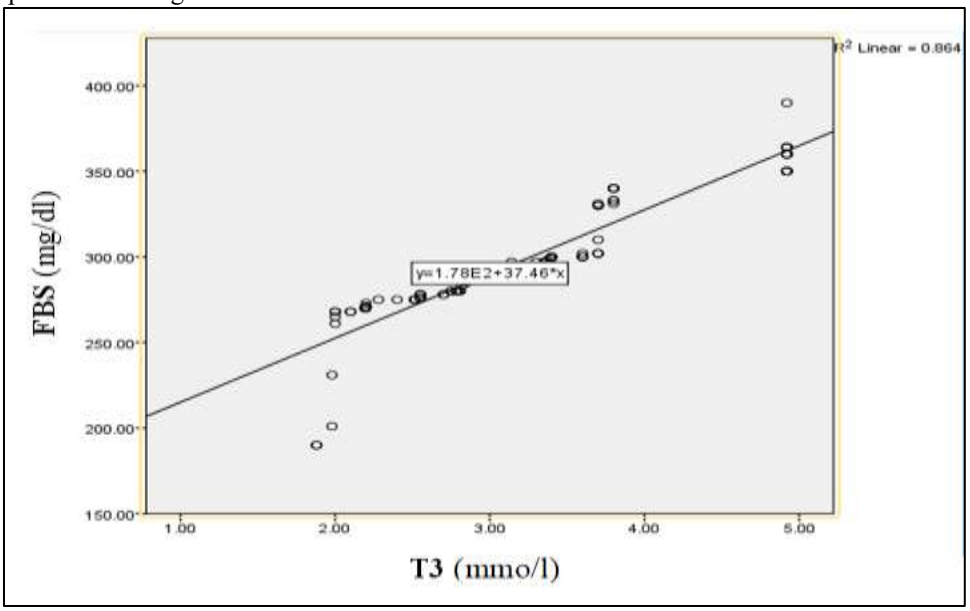

Figure 7. The correlation between T3 and FBS level in type IIDM patients complicated with hyperthyroidism.

The rising in LNFP was positively corelated (R^2=0.791) with the increasing in serum level of FBS (figure 8).

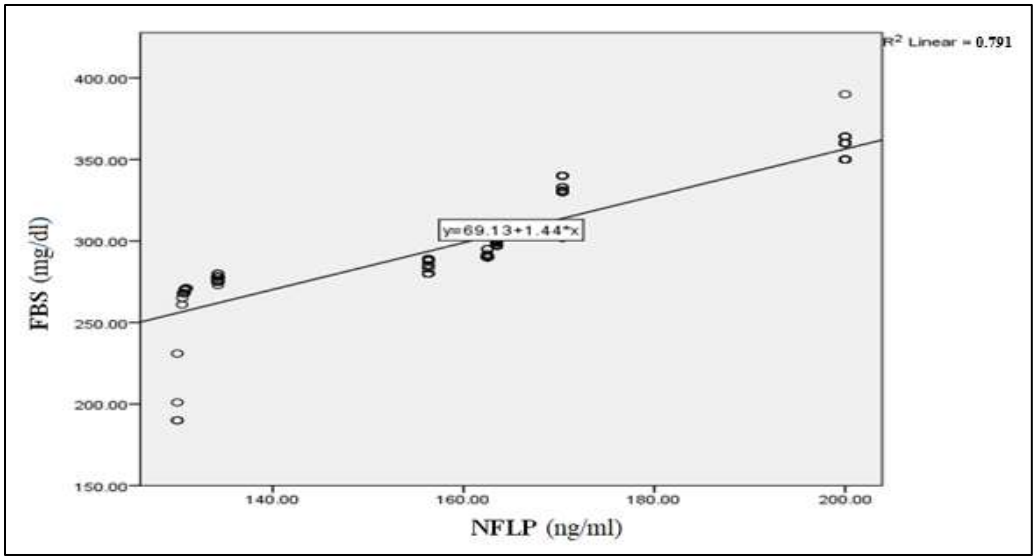

Figure 8: The correlation between serum LNFP and FBS in T2DM patients complicated with hyperthyroidism (108 patients).

In Figure 9, the correlation between the elevation in NFLP and T4 was weak (R2= 0.458) in patients with type II DM and thyroid dysfunction.

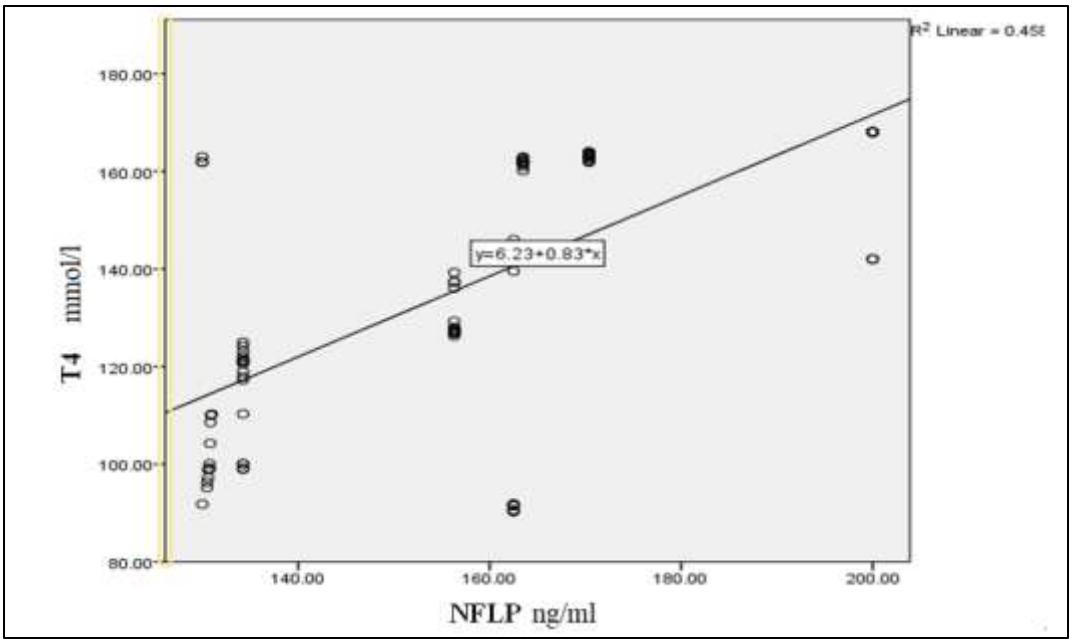

Figure 9: The correlation between NFLP and T4 in 108 patients with type II DM and thyroid dysfunction.

The elevating in serum light neurofilment protein was strongly (R^2=0.933) associated with the increasing in serum T3 as in figure 9.

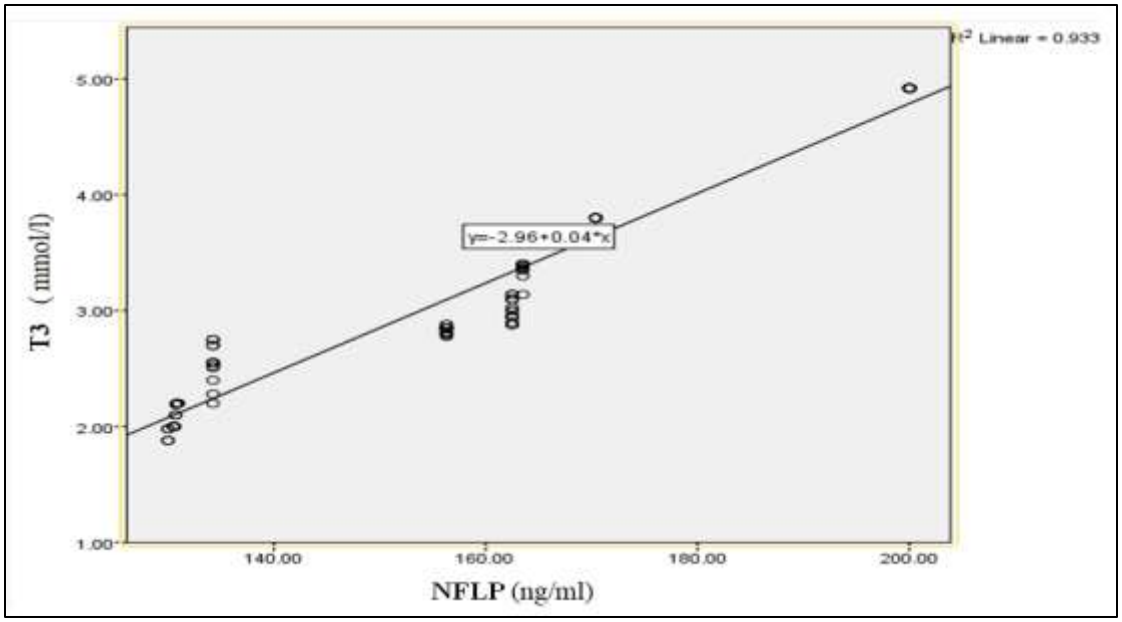

Figure 10: The correlation between NFLP and T3 in 108 patients with type II DM and thyroid dysfunction.

DISCUSSION

Over activity of thyroid gland causing hyperthyroidism (Grave's disease) and Low level of thyroid stimulating hormone, which can be associated with insulin resistance and poor glycemic management casing hyperinsulinemia also, abnormal thyroid function and thyroid tissue proliferation can effect on different site in body like pancreas, liver gastrointestinal tract, skeletal muscles, adipose tissue and central nervous system [14,15]. High level of T3 strongly was correlated with the increasing in FBS level (R^2 Linear = 0.864) but T4 didn't related with FBS level (R^2 Linear = 0.299). T3 is the only thyroid hormone form can inter cell, actually in pancreas its causing increase β cell proliferation and stimulated insulin secretion [15]. In liver thyroid hormone T3 increases hepatic gluconeogenesis by increase the activation of phosphoenolpyruvate carboxyl kinase (PEPCK) [16,17]. In addition, T3 can activate enzymes of glycogenolysis and induce hyperinsulinemia through peripheral insulin [18]. NFLP elevated in both T2DMHT and T2DM and its level associated with the degree of hyperglycemia because hyperglycemia and insulin resistance decrease synthesis of brain derived neurotrophic factor which responsible of neuroplasticity and cognitive. Also, in chronic hyperinsulinemia there were hyper activity in the insulin receptor substrate 1 leads to the insulin resistance and complicated with mitochondrial dysfunction, defective neurogenesis, protein aggregation oxidative stress, and inflammation [18- 20]. LNFP concentration increased significantly in T2DMHT as compared withT2DM patients, thyroid hormones regulation is very important for nervous system health, regulation expression many genes involved in many neurological process, neural cell proliferation and differentiation, migration. [21] However, FBS and T3 related with increased levels of NFLP, whereas no effect is exerted by T4. T3 hormone can affect neuron cells through its retrogradely transported in the sensory neurons soma, atomized motor and binds to specific intracellular receptor named thyroid hormone receptor beta isoform (TRβ) to activated gene expression of neurotrophic factors and cytoskeletal proteins. Also, T3 acts on Schwann cells in proximal through increase its proliferation and gene expression of molecules responsible of outgrowth of axotomized [22] Hyperthyroidism is associated with specific symptoms such as weight loss, palpitation and heat intolerance, cognitive dysfunction, various neuropsychiatric manifestations depression, dementia, mania, and encephalopathy, mean there were a resistance to thyroid hormones action because defect in the synthesis of TRβ , its express mutant TRβ [23,24] . The increase in T3 levels promote the increase in neurofilament protein aggregation and induced the proliferation of microglia and astrocytes those complications lead to poor management, mitochondrial damage, hyper inflammation, oxidative stress and then cause neurodegenerative disease [24-26]. Hypothalamic –pituitary-thyroid (HPT) axis affected by neurodegenerative process, TRH neurons in hypothalamus reduces stimulation of the anterior pituitary, causing low level of TSH also the inflammation that triggered by neurodegeneration can inhibit TRH neuron and finally effect on thyroid function { 27].

CONCLUSION

Neurodegenerative status was related to uncontrolled hyperglycemia and insulin resistant but its more aggressive in type II DM patients with hyperthyroidism, NFLP strongly related to toxic level of T3.

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