

# Antidiabetic Drugs Influences of the Activity of Acetylcholinesterase in Type 2 Diabetic Mellitus

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## Abstract

Many studies showed that several diseases including diabetes mellitus DM are directly implicated in the elevating of acetylcholinesterase AChE activity in most biological fluids and considered as a risk factor for neuronal death causing many degenerative disorders involving dementia. The most common dementia lesions related to DM are Alzheimer's disease AD. Metformin is currently as a first-line oral therapy for T2DM and considered a prophylactic factor to prevent of diabetic development. It lowers the AChE efficacy , that directs for the neurotransmitter ( Ach) dissociation. The current investigation involves a study the effect of metformin on the activity of AChE and some clinical variables for T2DM patients taken metformin monotherapy and combined to insulin of Iraqi population.

**Methods** : study population was 65 patients with type 2 diabetes and 22 healthy control subjects were age (42-80) year. The patients groups were distributed in three groups. The first had newly disease diagnosis without therapy , second had orally metformin monotherapy and the third had injected insulin with orally metformin as a combination therapy. The activity of acetylcholinesterase AChE and clinical biochemical tests ( FBG, HbA1c% , BMI , and lipid profile ) were assessed . Duration of disease and therapy and body mass index had been obtained from patients directly during their attending to the National Center of Diabetes in AL-Mustansyria University in Baghdad / Iraq.

**Results**: No significant difference in the AChE enzyme, BMI, TG, therapy duration and significant difference in age, FPG, HbA1c%, disease duration, T.CH, HDL, and LDL.

**Conclusion**: it was concluded that metformin drug lowers the activity of enzyme to the extent that had been appeared close to the normal activity values enzyme for healthy people in either monotherapy or combined therapy. As well as, it was more efficacy in male than female at all the studied variables for this investigation.

**Keywords** : Acetylcholinesterase Enzyme, Diabetes Mellitus, Dementia , Insulin, Metformin.

## INTRODUCTION

Diabetes mellitus DM is a chronic metabolic defect that is known as a hyperglycemia with disorders in carbohydrates, fats and proteins metabolism created from the deficiency in insulin releasing, insulin efficacy or both, appears as a consequence of many complicated actions of genetic, ecological agents , and lifestyle options [1] , [2]. Morbidity and mortality caused by DM are rising because of unhealthy demeanor like : unhygienic diet , physical inactivity , excess in weight , obesity and smoking [1]. Based on the published statistic evaluation of the International Diabetes Federation (IDF) in 2015, about 415 million adults are suffering DM globally. This number is predicted to reach 642 million in 2040 , which reflects that every 11<sup>th</sup> person will be diagnosed as T2DM [1] , [3]. According to the Diabetic American Association , DM was categorized into four mainly types depending on the disease causes , symptoms , and age of injury that includes type 1 (T1DM) , gestational GDM , type 2 (T2DM) , and other types of DM [4].

Cognition is defined as the rational actions or a process of information acquisition and recognition via thought , feeling , practice and proficiency [5]. The cognition decline or more precisely term the neurocognitive dysfunction [6] can be seemed as a consequence of DM even at early stages of disease[7] and chronic complications, that is recognized as the fourth diabetic microvascular complications [6], [8]. It is a vast range that have many domains [8], [9] , dilates from a mild cognition decline ( disability without difficulty performance of daily actions ) to intense dysfunction as a

dementia[5], [9]. It is generated from metabolic syndrome and cardiovascular disturbances that lead to modifications in the structure of brain and functional alterations at the central nervous system (CNS) [6] , [7] . Neurocognitive dysfunction is clarified as a reduction in entire cognitive domains included: learning , general memory and working memory , mental elasticity, attention , generic intelligence , somatosensory tests , executive functions , psychomotor and motor speed , verbal memory processing speed , instant recall or delayed calling , verbal glibness of vocabulary , visual retention, slowing gait speed , imbalance and increased falls [6] , [9]–[12]. DM is correlated to various risk agents that may inhibit the cognition roles , specific factors such hyperglycemia and hypoglycemia injuries within test , insulin deficiency , and insulin resistance , vascular factors that are linked to DM such hypertension , hyperlipidemia , obesity and microcomplication factors like vascular lesions (stroke) . In addition early starting of DM before the age of 65 , aging , insulin treatment , prolonged duration and acuteness of DM and others ( education , job , sex , depressive style , history of transitory ischemic attacks , substance abuse ) [10] , [12]–[14] . The most common dementia lesions related to DM are Alzheimer's disease AD [15] , [16] vascular dementia [16] and Parkinson disease PD [17] .

Indeed AD is counted as a microcomplication of DM due to the presence of the brain microvascular infarcts resultant from toxicity effects of hyperglycemia / hyperinsulinemia that reflect insulin signaling failure , which is related to the accumulation of pathological

causes and leading to brain atrophy and cognitive defects [8], [11], [18]. Acetyl cholinesterase (AChE, EC 3.1.1.7) is one of the most important enzyme in most living organisms, including humans and vertebrates, that catalyzes the dissociation of the neurotransmitter ACh, and thus finishing pulse transition, with a turnover number ( $10^8 \text{ s}^{-1}$ ) [19], [20] and the interaction rate is seen as a fastest of any other enzymes which breakdown each molecule in nearly 80 microseconds [21]. Several diseases are shared the (plasma, serum, RBC and leukocyte) increasing to AChE enzyme activity comprises hypertension, insulin resistance, dyslipidemia, DM and AD [22], [23]. As well many previous studies had been suggested that DM condition directly contributes to increasing of enzyme activity and considered as a risk factor for neuronal death in AD [24]. In DM and AD disorders a specified cells subsets and AChE isoenzyme can be impacted and lead to an elevating of the enzyme activity [22], [25] thus both the two diseases generally are most likely to manifest jointly and participate the existence of systemic and neuro inflammation [22], [26].

Metformin is currently as a first-line pharmacological oral therapy for T2DM widely in the obese and overweight patients with normal kidney and hepatic functions conditions in nearly all guidelines and testaments in the whole world [27]–[29].

This therapy reduces hepatic glucose production, which it has been appeared that is elevated (2-3) fold in disease status than normal condition. Further, it minimizes glucose digestion by intestine, and altitude insulin sensitivity by enhancing glucose uptake at peripheral tissues by means it improves the ability of insulin binding to their receptors at plasma membrane. This will occur with non-hypoglycemia incidence in either T2DM or normal cases and does not give rise to

#### Sample Collection

After getting patients official agreement, (10 ml) of vein blood in early morning were collected from each subject after 12 hour fasting, and divided into two parts. The first was put in gel plain tube for serum analysis, where the second was collected in the Ethylene Diamine Tetra Acetic acid (EDTA) anticoagulant tubes for HbA1c test. The study was approved by the institutional ethics committee.

### MATERIAL AND METHODS

#### Material

Kits of fasting blood glucose FPG and lipid profile including cholesterol TC, triacylglycerol TG, high density lipoprotein cholesterol HDL were used by (Bio Systems / BARCELONA / SPAIN) manufacture source. Glycated hemoglobin (HbA1c%) kit from (Integrated Sciences / AUSTRALIA). ELISA kit of serum activity of acetylcholinesterase enzyme (AChE) from Cusabio / CHINA company.

#### Study population

The study population consisted (65) of T2DM patients and (22) healthy control people were attending the National Center of Diabetes in AL-Mustansyria

cause hyperinsulinemia, in addition it diminishes the insulin resistance, especially in the liver and skeletal muscle [28], [30]–[33]. Metformin is considered a prophylactic factor to prevent or retard of diabetic development in the impaired glucose tolerance patients [33]. Most of the studies appeared that metformin using is related with significantly lowering the risks of cognitive impairment and thus incidence of dementia mostly AD in T2DM patients. Furthermore the oxidative stress weaken and normalizing at the hippocampus of brain and positives impacts on the function of the spatial memory, when has been compared to patients who are administrated another antidiabetic remedy [34]–[36]. Also there is a some proofs that it lowers the efficacy of AChE, that directs for the neurotransmitter (ACh) dissociation and contributes in the learning and memory roles [36]. The aims of the current study is to investigation the influence of metformin drug on the activity of acetylcholinesterase in diabetic patients, that implicated in the cognitive impairment, thereby how it attenuates the neurodegenerative disease like Alzheimer's disease AD.

#### Inclusion criteria :

The diagnosis of diabetes for all patients and control subject was carried out by physicians in the National Center of Diabetes in AL-Mustansyria University in Baghdad / Iraq. The inclusive criteria of the presented study was type 2 diabetes mellitus (T2DM) patients taken monotherapy of metformin drug or combined metformin with insulin treatment.

**Exclusion criteria:** Type 2 diabetes mellitus patients with renal failure diseases, patients were administrated any another antidiabetic therapy, woman T2DM pregnant patients and smoker patients.

University in Baghdad / Iraq during of interval January to March 2018. They were distributed in three different groups. Their age ranged (42-80) year. Twenty (20) patients had newly diagnosed in T2DM without treatment as group one. Second group had been monotherapy administrated metformin (22 sample) and the third group (23 patients) had been taken insulin combined with metformin as a therapy. Each patients group was consisted from nearly 50% of each gender male and female. Body mass index BMI was obtained immediately through weight and height, and history information were gained from patients after their agreement about their age, duration of T2DM, therapy duration, smoking and other diseases.

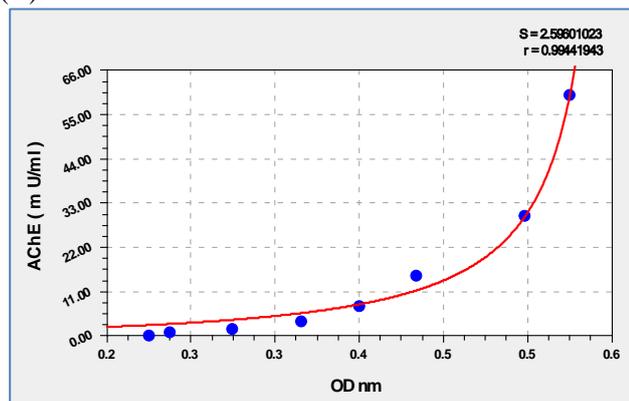
#### Clinical Test Assessment

All patients and healthy control subjects were clinically estimated for fasting plasma glucose FPG, glycated hemoglobin HbA1c%, serum lipid profile according to their manufacture instruction.

#### Acetylcholinesterase Activity (AChE) Assessment.

Serum activity of acetylcholinesterase enzyme was performed by quantitative sandwich type of enzyme-linked immunosorbent assay (ELISA) enzyme. Samples were diluted to 500-fold recommended serum dilution

and eight standard solutions were prepared according to assay instruction. The assay was applied and standard curve was obtained by plotting of optical density (OD) of standard solutions against their concentration, which appeared as a logistic curve by using the recommended curve Expert (1.3 version ) software program from manufacture instruction assay kit. The activity of ACHE (mU/L ) of sera samples were determined by plotting their OD at the Y –axis as illustrated in figure (1).



**Figure (1) :** Standard curve of human AChE activity by ELISA assay, X-axis represented the optical density (OD) in nanometer (nm) and Y-axis expressed the activity of enzyme of standard solutions expressed in (mU/ml). The statistical standard error and regression values were termed to *s* and *r* respectively .

#### Statistical Analysis

Statistical analysis was done by using computerize SPSS software program (version 20) , ONEWAY ANOVA to get differences between more than two groups and T-TEST statistical analysis for two independent groups programs at a significant difference

$P \leq 0.05$  level . The mean  $\pm$  standard deviation was termed to (Mean  $\pm$ SD) .

#### RESULTS AND DISCUSSION

The table (1) shows the (mean  $\pm$  SD) and ( *p*-value) of AChE enzyme activity and other clinical variables (age , BMI, FPG , HbA1c% , lipid profile , duration disease and therapy) .

#### Age / Duration Disease and Therapy Result

A significant statistical differences among total groups were appeared in age ( $P = 0.00465$ ) . Duration of diabetic disease was found to be significant difference ( $P < 0.00001$ ) among treatment groups . Meanwhile no significant variation revealed between therapy groups with regard of therapy duration .

#### Metformin Effect On The Acetylcholinesterase AChE Enzyme Activity

There was no significant statistical difference between patients groups and healthy control subjects ( $P = 0.082$ ) in the activity of AChE as shown in figures (2) . The diabetic situation involves the rising activity of acetylcholinesterase AChE , that is one of the causative factors for neuronal degeneration [24]. Several previous studies were done at induced diabetic rats demonstrated the rising activity of AChE enzyme in all brain composition domains which reflect the cognitive decline in this animal [37]–[39] . The researcher of this current study believed that unobvious distinctions in the AChE activity that was observed between normal control subjects and treated patients two groups as shown in figure (1), which denotes to the treatment T2DM by metformin lowers the activity of enzyme to the extent that had been appeared close to the normal activity values enzyme for healthy people.

**Table (1) :** The Mean  $\pm$  SD values of the activity of AChE enzyme and some clinical variables in patients and control healthy population of the study.

variables	Mean $\pm$ SD				P -values
	Control N= 21	Group 1 N= 20	Group 2 N=22	Group 3 N=23	
AChE u/ml	2.078 $\pm$ 0.9	2.18 $\pm$ 1.183	1.88 $\pm$ 0.4	2.75 $\pm$ 1.53	0.082
Age year	55 $\pm$ 7.7	56.8 $\pm$ 9.47	58.1 $\pm$ 7	62.9 $\pm$ 5	0.004651 *
BMI kg/m <sup>2</sup>	29 .13 $\pm$ 4	30.1 $\pm$ 5.7	31.2 $\pm$ 4.6	31.36 $\pm$ 4.95	0.405
FBG mg/dl	94.5 $\pm$ 10.3	154 $\pm$ 55.3	147 $\pm$ 52	193 $\pm$ 65	<0.00001 *
HbA1c %	5.4 $\pm$ 0.45	8.1 $\pm$ 1.6	7.5 $\pm$ 1.5	9.25 $\pm$ 1.7	<0.00001 *
T.CH mg/dl	180 $\pm$ 48	186.1 $\pm$ 52.2	152.5 $\pm$ 42.7	151.78 $\pm$ 53	0.00686 *
TG mg/dl	120 $\pm$ 90.6	148.4 $\pm$ 89.5	127 $\pm$ 83.9	137.5 $\pm$ 95.8	0.763
HDL mg/dl	52.7 $\pm$ 22.8	46.2 $\pm$ 18.2	40.89 $\pm$ 14.1	37 $\pm$ 11.96	0.0198 *
LDL mg/dl	114.8 $\pm$ 42.2	109.5 $\pm$ 42.3	86.27 $\pm$ 34.6	87.4 $\pm$ 35.6	0.029 *
Disease Duration		(7.1 $\pm$ 6.6) (Months)	6.1 $\pm$ 4.1 (years)	11.8 $\pm$ 4.45 (years)	< 0.00001 *
Therapy Duration (years)			5.3 $\pm$ 4.1	5.95 $\pm$ 4.78	0.329

\* Significant using ONEWAY-ANOVA and T-TEST at 0.05 level of significance.

- Control : indicate to healthy normal subjects which was used as a control group.
- Group 1 : indicate to newly detected diabetic patients group without treatment by any antidiabetic drug
- Group 2 : indicate to patients group who taken metformin as a monotherapy.
- Group 3 : indicate to patients group who taken metformin in a combination therapy with insulin.

The cause of non-existence of disparity between the newly detected patients group 1 and treated groups (2 & 3) was the small duration disease of group 1, that had only (7.1± 6.6) months, and the diabetic neuropathy complication appearance requires longer duration of disease.

Although the difference in activity of enzyme among group 2 (metformin monotherapy) and group 3 (metformin combination therapy) was statistically non-significant, but it observed there was a slight disparity between two groups as shown in figure (2). It was higher in group 3 than group 2, this might be due to duration disease for group 3 was longer (11.8± 4.45) than group 2 (6.1± 4.1) with a close treatment duration for the two treated groups as shown in table (1).

Several previous *in vivo* studies estimated the effect of metformin on AChE activity, for example (Bhutada et al. 2011) investigated the effect of metformin on cognitive function in induced diabetic rats by streptozotocin. The authors assessed activity of (AChE) as a marker for cholinergic roles. It was found that induction of diabetes in rats involved to a severe impairment in learning and memory related with increased AChE activity. Also was found that (30 days) treatment by a dose of 500 mg/kg of metformin enhanced cognitive performance and reduced the activity of AChE [57]. Another *in vivo* study was done by (Saliu et al. 2016) demonstrated that metformin at a dose of 500 mg/kg considerably decreased AChE activity at the brain for streptozotocin-induced diabetic rats [58].

#### **Metformin Effect On The Body Mass Index BMI**

Non-significant statistical difference between groups ( $P > 0.05$ ) result was shown in body mass index BMI. Metformin have the positive influences on the reducing BMI. It lowers insulin resistance, that is a mainly cause in diabetic disorder, further in obese non-diabetic person. Therefore improvement insulin sensitivity give rise to the decreasing of weight [40]. The result was agreed (Mohammed 2017) at the statistical side only, who found no significant differences ( $P = 0.077$ ) among Iraqi diabetic patients and healthy subjects groups, its result was a very slight decline in BMI for diabetic population study, which dropped from (24.3 ± 1.4) in control subjects to (24.2 ± 1.5) in diabetic patients [41]. The results of the current study appeared that BMI higher in the two treated groups (2 & 3) than group 1 (newly diagnosis), and control healthy subjects as shown in table (1). This indicate that metformin drug did not achieve the desired goal in the lowering BMI as in well known, due to the lifestyle or genetic factors that play an important roles in BMI.

#### **Metformin Effect On The glycemic control .**

##### **1. Fasting Plasma Glucose FBG**

Highly statistical significant increasing in FBG result ( $P < 0.00001$ ) was showed. This results was in agreement to (Bhatt and Lochan 2015) [42]. The uncontrolling on hyperglycemia was defined according to the recommendation of American

Diabetes Association (ADA), that is the fasting blood glucose (FBG) level is (250 mg/dl), random glucose level ( $\geq 300$  mg/dl), hemoglobin glycosylated ( $> 10\%$ ) or the present of ketonuria [43]. Good glycemic control must be ( $\leq 150$  mg/dl) [44], the level of FPG in group 2 (metformin therapy alone) achieved good glycemic control, which was (147 ± 52). Metformin minimizes hyperglycemia by inhibiting the hepatic glucose production and insulin resistance particular in liver and skeletal muscles [31]. In group 3 (met plus insulin) treatment patients, the level of FBG appeared an acceptable values ( $< 200$  mg/dl). Insulin therapy is the best choice for poor glycemic control [45] and recommended by ADA. It regulates glucose levels in plasma through the inhibition of glucose production and elevating the peripheral tissues activity absorption. Also it lowers the toxicity influences of glucose by reducing hyperglycemia and enhances insulin and beta cells releasing roles [44]. Since combination therapy of (metformin and insulin) has been appeared an advantage to ameliorate glycemic control more effective than administration insulin therapy alone [45].

##### **2. Glycated Hemoglobin HbA1c %.**

Hemoglobin glycosylated (HbA1c %) outcomes showed considerably statistical significant disparity increasing ( $P < 0.00001$ ). This test is widely used as a biomarker for glucose regulation at a long-term and it is more efficient than FPG test for a reason that it can be utilized for non-fasting condition test, as well as the stability of level glucose concentration. The test predicts the risks diabetic complications progression [46], [47]. Group 2 (met) patients of this study achieved a good control for HbA1c% (7.5 ± 1.56), which was close to preferred physiological level ( $< 7\%$ ) particular in the elderly patients. Tight or intensive glycemic control is in general regarded as the diet applying requires to a level of HbA1c % ranged (6.0 to 6.5)%. This level was recommended and proper for young, newly diabetic patients diagnosis with lower happening of cardiovascular risks, especially when they were initially administrated metformin as antidiabetic drug particular in obese and overweight patients [48]. In these patients the tight glycemic control occurs without hypoglycemic risks incidents. But in older patients with longer duration of diabetic disease and have the highly risk of cardiovascular. This intensive control glycemic was not globally recommended relying on the most researchers and specialist clinicians advises, due to the older patients are possible to have higher risk of morbidity because the rising risk of hypoglycemia and increasing the hazards negative side effects of drug. Hence patients who are at a high macrovascular complications should be reduced their level of (HbA1c %) slowly [48].

Group 3 (met & insulin) had been revealed that did not achieve the convenient glycemic control (9.2 ± 1.7)%, this is because to those patients had poorly

glycemic control which also the FBG level showed ( $193 \pm 64.97$ ). Therefore they were recommended to insulin therapy utilizing and also they had longer duration of disease ( $11.8 \pm 4.45$ ) year compared to group 2 who they were ( $6.1 \pm 4.1$ ) year and both the two groups had about therapy drug duration were close.

### Metformin Effect On The Lipid Profile

The findings of total cholesterol T.CH was found a highly significant reduction ( $P = 0.000686$ ) between the patients groups when compared to healthy normal control set. The result was in agreement to (Mouráo-junior et al 2006) who demonstrated a significant decreasing in the level of T.CH under metformin treatment during six months of administration this drug [49]. The current investigation showed variation between normal control group and treatment patients groups 2 and 3 and between these groups and group 1 (newly patients diagnosis), during the metformin therapy duration of the two treatment groups, that was explained in table (1), the reduction in the T.CH levels was 21% for group 2 and 21.4% for group 3 depending on the group 1.

This dropping is due to the metformin has beneficial effects on the lipid metabolism. A dose more than (1000 mg/day) of metformin is able to inhibition of bile acid uptake by intestine, which leads to minimize the serum bile acid, therefore increasing of liver bile acid generation. This path needs to cholesterol to be done and finally gives rises to hepatocytes reduction of cholesterol [50]. There was no distinction observed among group 2 and 3, indicate that treatment by metformin alone or mixed to insulin had the same effect on the T.CH levels as we mentioned above and this result was agreed to (Sharif and Younis 2017) [51]. There was no statistical significant variance showed between patients groups and healthy control subjects in the triglyceride TG levels ( $P > 0.05$ ). But the researchers believed that was a slight decline in TG concentration in groups 2 and 3 during the therapy duration were close of two groups that clarified in table (1). This dropping was found nearly 14.1% for group 2 (metformin) and 7.3% for group 3 (mixed metformin to insulin) depending on the mean TG levels of group 1 (newly diagnosis). The result was disagreed (Lin et al 2018) for a statistical point of view but was agreed with them from the slight reduction side. This researcher found that it reduction was dropped from ( $132 \pm 71.9$  mg/dl) to ( $122 \pm 63.6$  mg/dl) during therapy duration of metformin monotherapy was one year and it was explicated to "diminishing hypertriglyceridemia requires a longer therapeutic duration to counteract than lowering LDL" [52].

The outcome of high density lipoprotein cholesterol HDL revealed that was significant decreasing ( $P = 0.0198$ ) among patients and control groups. No difference was noticed between three group patients (1, 2, and 3). This refers to the metformin therapy had no impact on the HDL concentration [53]. The result

was in agreement to (Mouráo-junior et al 2006) [49], the diversity was clear and statistically significant between the control group and treatment patients groups (2 and 3). This indicates to diabetic dyslipidemia that was known a lipid metabolic syndrome including lowering levels of HDL [50] and according to the National Cholesterol Education Program's Adult Treatment Panel III was limited the HDL concentration less than 1.3 mmol/L ( $< 50$ mg/dl) in women and less than 1.03 mmol/L ( $< 40$ mg/dl) in men [54]. The result of the current study was agreed (Yassin and Al-kayatt 2011) who found "the HDL concentration in T2DM patients group was lower than nondiabetic non-obese control group but it shows no significant difference" [55]. Also our result appeared no difference between therapy groups (2 and 3) and this finding was concordant to (Sharif and Younis 2017) [51].

The low density lipoprotein cholesterol LDL results showed highly significant difference ( $P < 0.00001$ ) between patients and normal healthy groups. The variances were appeared among all groups except group 1 (newly diagnosis) patients, which may be interpreted to the small disease duration for group 1 that was only ( $7.1 \pm 6.6$ ) months as shown in table (1). The result was in concordance to the findings of (Lin et al 2018) [52], and (Yassin and Al-kayatt 2011) [55]. The level of LDL was lowered almost 21.2% and 19.8% depending on the group 1 (newly diagnosis) level for (2 and 3) groups respectively. The reason of this dropping can be explained to ameliorating the sensitivity of insulin by metformin drug, leading to prevent lipid accumulation at the skeletal muscle through promoting fatty acid oxidation [56]. Further there was no difference between group 2 and group 3, this result was agreed (Sharif and Younis 2017) [51].

### Metformin Effect On The Gender

The T-Independent statistical analysis test was applied to study the drug impact on two gender for AChE enzyme activity and other clinical parameters of the two treated groups. Tables (2) explain ( $m \pm SD$ ) for control and newly diagnosis patients group and table (3) for two treatments group 2 and 3.

The results revealed that no significant difference between two genders in the activity of AChE enzyme, which reflect metformin drug had the same decreasing effect in male and female for the two types of treatment (combination and monotherapy of metformin) as shown in the figure (3), however the authors believed that a slight non-significant difference was clear between male and female in the two treated groups (2 & 3). For combination therapy the male was found ( $2.44 \pm 0.961$ ) less than female ( $3.1 \pm 1.98$ ), and in monotherapy showed in male ( $1.78 \pm 0.335$ ), but in female was ( $1.97 \pm 0.452$ ). This indicate that the activity of enzyme in male was lowered by metformin more than female.

Levels of T.CH for group 1 (newly) and control group showed no significant difference among male and

female as shown in table (3.8), but there was significant reducing difference between two genders for both treated groups, which indicate the monotherapy of metformin and combination metformin with insulin had different influence on two genders. Metformin monotherapy appeared that T.CH reduced nearly 9.4 % for female sex, but in male was 26.8 % . Meanwhile mixed metformin and insulin therapy appeared that the reducing of T.CH in female about 6.1% and in male 29.78 % . This result refers to metformin drug as a monotherapy or combined therapy more effective in male than female .

The levels of LDL showed no statistical significant differences among both sex for the control healthy subjects and group 1 (newly) as shown in tables (2) , but there were statistical significant differences showed between genders in group 3 (met& insulin) , which it lowered approximately 3. 84 % in female and in male was 35.1 % . This indicate combination therapy of metformin had different effect on male and female The differences between two gender in group 2 (metformin monotherapy) appeared no statistical variations in LDL levels (P= 0.066) , but we believe there was a slight difference among male and female as shown in table (3).

Levels of FBG revealed that no significant difference in male and female for control , newly diagnosis , and metformin groups as shown in tables (2) and (3), but in group 3 (met & insulin) showed significant difference , which reflect to different effect on two sexes , It was found relatively good glycemic control on male gender ( $158.4 \pm 41.78$ ) , but in female appeared a poor glycemic control ( $>150$  mg/dl) during the mean duration therapy was ( $5.95 \pm 4.78$ ) . This indicates that combined metformin treatment more efficiency in male than female. Meanwhile metformin monotherapy had a good glycemic control (FBG levels) in both gender ( $<150$  mg/dl) during the therapy duration ( $5.36 \pm 4.1$ ), which appeared no statistical significant difference among men and women (P= 0.936) as shown in table (3) .

Other clinical variables ( HbA1c % , BMI and HDL) showed non-significant statistical differences among men and women (P > 0.05) for two studied treated groups , but TG level appeared significant statistical variation among two sexes in control healthy subjects and in three patients groups found no significant statistical disparity as explained in table (2) and (3) .

Table (2): the (mean  $\pm$ SD) values in male and female for control healthy and newly diagnosis patients groups.

variable	MEAN $\pm$ SD CONTROL GROUP			MEAN $\pm$ SD GROUP 1 NEWLY		
	CF	CM	P	G1F	G1M	P
AChE U/ml	2.13 $\pm$ 0.94	2.03 $\pm$ 0.866	0.82	2.18 $\pm$ 1.07	2.19 $\pm$ 1.35	0.98
BMI Kg /m <sup>2</sup>	28.9 $\pm$ 3.7	29.3 $\pm$ 4.4	0.81	32.1 $\pm$ 6.9	28.2 $\pm$ 3.6	0.13
FBGmg /dl	91.3 $\pm$ 9.8	97.9 $\pm$ 10.2	0.14	149.7 $\pm$ 62.7	158 $\pm$ 50	0.73
HbA1c%	5.3 $\pm$ 0.38	5.4 $\pm$ 0.55	0.65	7.94 $\pm$ 1.87	8.3 $\pm$ 1.44	0.63
CH mg /dl	180.6 $\pm$ 55	208.3 $\pm$ 36.3	0.19	188.7 $\pm$ 49.4	183 $\pm$ 57.4	0.83
TG mg /dl	81.9 $\pm$ 57.6	162.6 $\pm$ 103.8	<b>0.038*</b>	170.7 $\pm$ 108.2	126.1 $\pm$ 64.1	0.27
HDL mg /dl	60.8 $\pm$ 28.3	43.9 $\pm$ 10.1	0.091	43.77 $\pm$ 13.1	48.7 $\pm$ 22.6	0.55
LDL mg /dl	104.9 $\pm$ 50.4	125.8 $\pm$ 29.6	0.26	109.3 $\pm$ 43.4	109.7 $\pm$ 43.4	0.98

\* Significant using T-TEST at 0.05 level of significance.

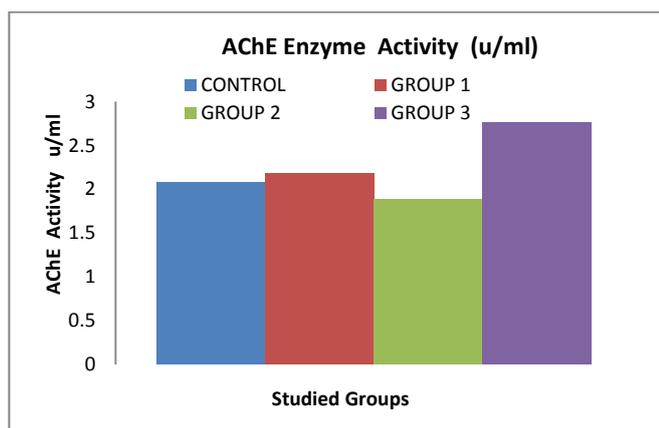
CF : indicate to control female , CM: indicate to control male, G1F and G1M refer to female and male newly diagnosis patients groups respectively.

Table (3): the (mean  $\pm$ SD) values in male and female for group 2(met) and group 3(met & insulin)

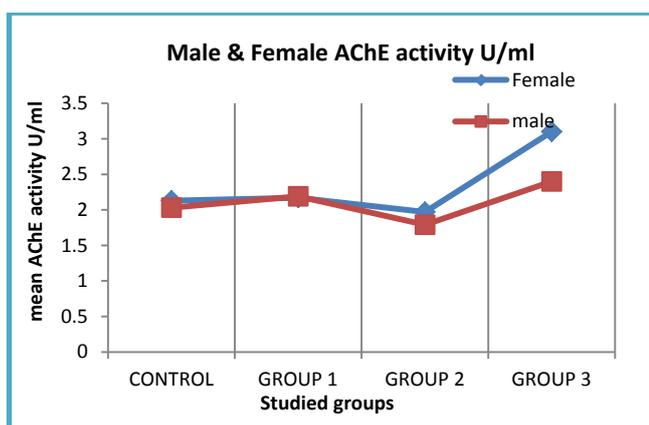
Variable	MEAN $\pm$ SD GROUP 2 (METFORMIN)			MEAN $\pm$ SD GROUP 3 (METFORMIN & INSULIN)		
	G2F	G2M	P	G3F	G3M	P
AChE U/ml	1.97 $\pm$ 0.452	1.78 $\pm$ 0.335	0.34	3.1 $\pm$ 1.98	2.44 $\pm$ 0.961	0.338
BMI Kg /m <sup>2</sup>	31.9 $\pm$ 5.95	30.5 $\pm$ 2.87	0.49	33 $\pm$ 5.85	29.83 $\pm$ 3.66	0.126
FBG mg /dl	146.7 $\pm$ 36.2	148.6 $\pm$ 67.7	0.936	230.8 $\pm$ 66	158.4 $\pm$ 41.78	<b>0.045*</b>
HbA1 c%	7.39 $\pm$ 1.05	7.62 $\pm$ 1.99	0.74	8.87 $\pm$ 1.75	9.59 $\pm$ 1.65	0.322
CH mg /dl	171 $\pm$ 37	133.9 $\pm$ 41.3	<b>0.038*</b>	177.2 $\pm$ 61.08	128.5 $\pm$ 31.97	<b>0.0243*</b>
TG mg /dl	154 $\pm$ 93.5	99.91 $\pm$ 66.56	0.133	172.36 $\pm$ 118.8	105.67 $\pm$ 56.7	0.095
HDL mg /dl	40.55 $\pm$ 12.7	41.25 $\pm$ 16	0.91	37.64 $\pm$ 9.015	36.42 $\pm$ 14.55	0.813
LDL mg /dl	99.73 $\pm$ 33.87	72.8 $\pm$ 31.2	0.066	105.1 $\pm$ 36.36	71.17 $\pm$ 21.16	<b>0.0186*</b>

\* Significant using T-TEST at 0.05 level of significance.

G2F : indicate to female group 2(met) ,G2M: indicate to male group 2(met) ,G3F and G3M refer to female and male group 3(met & insulin) respectively.



**Figure (1) :** Histogram figure for the activity of AChE enzyme for patients and control groups. Control refers to normal healthy subjects of enzyme activity, Group 1 : newly diagnosis without therapy patients, Group 2 : patients had been administrated metformin as a monotherapy and Group 3 : patients had been taken combined metformin therapy to insulin .



**Figure (3.12) :** AChE activity in male and female for three patients and control groups.

### CONCLUSION

Metformin had been and still the first and gold standard choice of treatment for type 2 diabetic patients . it provides more valuable effects due to its safety and efficiency mostly as a protective factor for the neurodegenerative disease and cognitive dysfunction.

In summary it was concluded that metformin drug lowers the activity of enzyme to the extent that had been appeared close to the normal activity values enzyme for healthy people in either monotherapy or combined therapy. As well as, it was more efficacy in male than female in all the studied variables for this investigation.

### Acknowledgments

We are grateful to the National Center for Diabetes of AL-Mustansyria University in Baghdad / Iraq especially to MSC Biochemist Bayda'a Ahmed . Also the authors are grateful to AL-Razi Center / Corporation for Research and Industrial Development / Iraqi Ministry of Industry

& Minerals especially to MSC Biologist Wesal Hisham and to Veterinary Doctor Samira .AL- azawi .

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