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RESEARCH ARTICLE

Evaluation of some risk factors affecting insulin resistance in diabetic patients

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Manuscript Info *Abstract*

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BACKGROUND: The higher prevalence of Diabetes mellitus(DM) and Insulin resistance(IR) in patients with Hepatitis C Virus(HCV)infection was accompanied with increased expression of Cytochrome P450 2E1 (CYP2E1). This study was designed to evaluate the relation between diabetes and HCV infection by estimating some risk factors (body weight, age & sex), biochemical parameters (Tumor Necrosis Factor Alpha (TNF-α) level, lipid profile, Glutathione reduced(GSH) concentration, liver Aspartate Amino transferase (ASAT) activity, liver Alanine Amino Transferase (ALAT) activity,Creatinine level, blood Urea nitrogen (BUN) level) and molecular parameters. **Methods:** Fifty patients were divided into five groups each containing ten patients Group**1;** Control group included diabetic patients with normal insulin resistance level, **Group 2;** included diabetic patients with high level of insulin resistance, **Group 3**; included diabetic patients with high level of insulin resistance & positive hepatitis c virus, **Group 4**; included diabetic patients with high levels of both insulin resistance and TNF-α, **Group 5;** included diabetic patients with high level of insulin resistance and have coronary heart diseases. **Results:** Patients of diabetic HCV infected-group were significantly older and had higher serum ASAT and ALAT levels ($P<0.01$). The presence of hepatitis c virus in the diabetic patients in group (3) (gp.3) made a highly significant increase in the insulin resistance in compared to the control group(1), diabetic HCV patients have significantly high of soluble TNF-α ($P<0.01$) and in BUN levels in compared to control group (1), presence of hepatitis c virus in the diabetic patients did not show a significant change ($P>0.05$) in creatinine level in comparison to control group (1),a highly significant reduced levels of serum GSH, increased expression of CYP2E1 in HCV infected patients. **Conclusion:** Diabetic patients with HCV infection showed a significantly higher level of IR , TNF-α and increased expression of Cytochrome P450 2E1 (CYP2E1) than those without HCV infection.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by high blood sugar (glucose) levels that result from defects in insulin secretion, or action, or both. Elevated levels of blood glucose (hyperglycemia) lead to spillage of glucose into the urine, hence the term sweet urine **(Fox *et al*.,2015).**

Insufficient production of insulin or the inability of cells to use insulin properly and efficiently leads to hyperglycemia and diabetes. This latter condition affects mostly the cells of muscle and fat tissues, and results in a condition known as "insulin resistance". This is the primary problem in type 2 diabetes.The absolute lack of insulin, usually secondary to a destructive process affecting the insulin producing beta cells in the pancreas (**Boussageon*et al*., 2011)**.

The highest Hepatitis C Virus (HCV) prevalence in the world occurs in Egypt at an estimated 12% among the general population and reaches 40% in 40 years old persons and above in rural areas **(Mastoi et al., 2010)**. HCV Genotype 4 is the predominant genotype being isolated from up to 91% of HCV-infected persons in Egypt. The origin of the HCV epidemic in Egypt has been attributed to intravenous schistosomiasis treatment in rural areas in the 1960s-70s **(Toth, 2005).**

Type 2 DM begins with insulin resistance, a condition in which cells fail to respond to insulin properly. As the disease progresses a lack of insulin may also develop (**Shi*et al*., 2012)**. It is a major public health problem worldwide (**Tabish, 2007**). A number of lifestyle factors are known to be important to the development of type 2 diabetes, including obesity (defined by a body mass index of greater than thirty), lack of physical activity, poor diet, stress, and urbanization.**(Shlomo*et al*., 2011).**Several studies from different parts of the world have found that 13% to 33% of patients with chronic HCV have associated diabetes, mostly type II DM (**Chehadeh*et al*.,2009).** Diabetes was more frequent in patients having liver cirrhosis than those having chronic hepatitis **(Loaeza-del Castillo, 2005).**

Tumor necrosis factor-alpha (TNF-α) is a 157-amino acid pro-inflammatory cytokine predominantly produced by monocytes, macrophages, and T-cells. TNF-α is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. It is produced chiefly by activated macrophages (M1), although it can be produced by many other cell types as CD^{4+} lymphocytes, NK cells and neurons **(Swardfager*et al.*, 2010)**.

The cytochrome P450 enzyme family of heme-containing proteins represents one of the largest and most functionally diverse superfamilies found in nature (**Nelson *et al*., 2004**).Hepatitis C virus(HCV) infection was reported to cause changes in levels of drug metabolizing enzymes cytochrome P450 2E1 (**Gochee et al., 2003)**which is an oxidative stress-related enzyme (**Levent *et al*., 2006**).

Subjects and methods:

Subjects:

This study was done in Ain Shams University Specialized Hospital (ASUSH). Data was collected from diagnosed diabetic patients having followed up at the hospital diabetic clinic.

All patients were subjected to full clinical examination including history talking and vital data (blood pressure measurement, The age, gender, height, weight and body mass index (BMI)))will recorded**.** Blood samples were collected for fasting serum glucose level, albumin level, liver enzymes (ALAT, ASAT) activity, creatinine level, blood urea nitrogen level, lipid profile and glutathione reduced concentration determination, using an auto-analyzer in Ain shams specialized hospital lab. The aliquots of separated serum were frozen at -80°C for insulin and TNF-α level measurement. The incidence of HCV infection will be detected by serum real time polymerase chain reaction (RT- PCR, Roche Diagnostic System), also the extraction of total RNA from blood and detection of expression of CYP2E1 gene.

Fifty patients were included in the study and classified into 5 groups**:**

Group 1: Control group included ten diabetic patients with normal insulin resistance level.

Group 2: Included ten diabetic patients with high level of insulin resistance.

Group 3: Included ten diabetic patients with high level of insulin resistance & positive hepatitis c virus.

Group 4: Included ten diabetic patients with high levels of both insulin resistance and TNF-α.

Group 5: Included ten diabetic patients with high level of insulin resistance and have coronary heart diseases.

The index of insulin resistance will be calculated on the basis of fasting values of plasma glucose and insulin, according to the homeostasis model assessment (HOMA) method, HOMA = (glucose in mmol/L × insulin in μU/mL/22.5)(**Enzo et al., 2002**) .

Methods:

A-Determination of Hepatitis C Virus (HCV):

The incidence of HCV infection was detected using The COBAS AmpliPrep/COBAS TaqMan HCV Test according to **(Sizmann *et al*., 2007).**

The COBAS AmpliPrep/COBAS TaqMan HCV Test is based on three major processes:

1-**Specimen preparation to isolate HCV RNA,** Selection of the target RNA sequence for HCV depends on identification of regions within the HCV genome that show maximum sequence conservation among the various HCV genotypes. (**Kuiken*et al* ., 2005**).

2-Reverse Transcription and PCR Amplification:

The reverse transcription and PCR amplification reaction is performed according to **(Smith *et al*., 2003).**

3-Detection of PCR Products in a COBAS TaqMan Test:

The COBAS AmpliPrep/COBAS TaqMan HCV Test utilizes real-time PCR technology according to **Smith et al., 2003.**

B-Reverses transcription PCR (QRT-PCR) for gene expression analysis.

Much fluorescent chemistry was used to detect gene transcripts. One method for real-time uses SYBR® Green I, a dye that fluorescence when bound nonspecifically to double-stranded DNA. The Brilliant II SYBR Green QRT-PCR Master Mix with ROX, 1-Step kit includes the components necessary to carry out cDNA synthesis and PCR amplification in one tube and one buffer.

C-Determination of Tumor Necrosis Factor Alpha (TNF-α):

TNF-α was determined by using The Human Tumor Necrosis Factor-Alpha UltraSensitive (Hu TNF-α US) ELISA kite **(Atègbo,2006)**

Statistical analysis:

The data obtained were analyzed by one-way analysis of variance and t-Student test for the significant interrelation between the various groups using (**Turner, 2001**) GraphPad prism version 5 for windows, GraphPad software (San Diego, CA, USA). Probability levels of more than 0.05 were considered insignificant ($P>0.05$), while less than 0.05 were considered significant ($P<0.05$), while less than 0.01 were considered highly significant ($P<0.01$), less than 0.001 were considered very highly significant ($P<0.001$).

Results:

The mean ± SD of diabetic patients with insulin resistance (54.4 ± 7.18)(group 2) did not induce ($P<0.05$) any significant change of the Age of patients as compared to control (group 1) also the HCV incidence (52.6 ± 3.53), increase in tumor necrosis factor (54±7.30) and incidence of coronary heart disease(51.7 ±6.09) did not induce ($P<0.05$) any significant change of the Age as compared to diabetic patients with insulin resistance (group 2). While **the mean ± SD** of diabetic patients with insulin resistance (**30.17±1.55)** was highly significant increased as compared to control group (1), while presence of HCV infection (31.65± 1.11) and increased tumor necrosis factor (31.6±0.96) did not induce any significant change on the contrary the incidence of coronary heart disease resulted in a significant elevation in the mean **± SD**(31.87±1.45).

The mean ± SD (207.5±36.23) of diabetic patients with insulin resistance showed a very highly significant elevation in the level of blood glucose as compared to control group (gp.1). On the contrary

There is not any significant change in the **mean ± SD** of HCV -infected group (gp.2) (191.2 **±** 13.67), increased level of tumor necrosis factor –group (gp.4) (191.5 **± 39.47) and coronary heart disease infected group (gp.5) (232.7±45.41).**

The mean ± SD (29±1.05) of diabetic patients with insulin resistance indicated a very highly significant increase in the insulin level in comparison with control group (gp.1), while did not show any significant change in the level of insulin in the HCV –infected group (gp.3)(28.7±3.47) as compared to diabetic patients with insulin resistance (gp.2).on the contrary , The mean ± SD (15.7± 3.86)of the increased tumor necrosis factor–group (gp.4) showed a very highly significant decline as compared with diabetic patients with insulin

resistance (gp.2).while the mean ± SD (49.5±6.04) of coronary heart disease - group with diabetes mellitus revealed a very highly significant increase (P<0.001) in the fasting insulin level as compared to diabetic patients with insulin resistance (group 2).

The mean ± SD (14.89±2.86) of diabetic patients with insulin resistance indicated a highly significant increase in the HOMA index in comparison with control group (gp.1), while the mean ± SD (13.59±2.38) of HCV-infected group did not show any significant change as compared with group (2) on the contrary, The mean ± SD of the increased level of tumor necrosis factor group showed a very highly significant decline (7.92±7.32) as compared with group (2); The mean ± SD (28.39±6.69) of coronary heart disease revealed a very highly significant increase in the HOMA index as compared to group (2).

The mean ± SD (3.92±0.26) of diabetic patients with insulin resistance did not show any significant change in the level of albumin as compared with control group (group 2); on the contrary, the mean ± SD (3.02±0.19)of HCV-infected group showed a very highly significant decline as compared with diabetic patients with insulin resistance (gp.2), while the mean ± SD (0.25±0.50) of the increased level of tumor necrosis factor-group and mean ± SD (0.22 ± 0.47) of
Coronary heart disease group if compared with **diabetic patients with insulin resistance (gp. 2).**

The mean ± SD (23.5±2.22) of diabetic patients with insulin resistance did not show any significant change in the activity of liver Alanine Amino Transferase in comparison to control group (gp.2),**the mean ± SD (3.92±0.26)** on the contrary , **The mean ± SD (78.±0.26)** of HCV infected –group revealed a very highly significant increase when compared with group (2) ,while **The mean ± SD (**22.8±**6.015**) of increased level of tumor necrosis factor-group and coronary heart disease-group (27.8±5.28) did not induce any significant change in the activity of liver Alanine Amino Transferase in comparison with group(2).

The mean **± SD (22.2±1.69)of diabetic patients with insulin resistance** did not show any significant change in the activity of liver aspartate amino transferase when compared with control group (gp.1), On the contrary the mean **± SD (36.6±4.86) of hcv-infected group (gp.2) showed a very highly significant** increase in the activity of liver aspartate amino transferase as compared to group 2(gp.2) ,while The mean **± SD (25.6±7.60)** of increased level of tumor necrosis factor-group and of coronary heart disease -group (21±3.527) did not reveal any significant change when compared with group(2).

The mean **± SD (19.2±1.66)of diabetic patients with insulin resistance did not induce any significant change** in level of BUN when compared to control group (gp.1), while the mean ± SD (18±2.87) of HCV-infected group revealed a very highly significant increase in the level of BUN when compared with group (2), while The mean **± SD (13.2±2.10)of** increased tumor necrosis factor-group did not induce any significant change in the level of serum BUN when compared with group (2),on the contrary, the mean **± SD (17.6±3.47)** of coronary heart disease –group showed a highly significant elevation in the level of BUN when compared with group (2).

The mean **± SD (0.87±0.15)of diabetic patients with insulin resistance did not induce any significant change** in level of creartinine when compared with control group (gp.1), also the mean ± SD (0.96±0.36) of HCV-infected group did not show any significant change in the level of creatinine when compared with group (2) also, the mean **± SD (0.80±0.17)** of increased tumor necrosis level-group when compared with group (2), on the contrary, the mean **± SD (1.04±0.18)** of coronary heart disease –group showed a highly significant elevation when compared with group (2).

The mean **± SD (176.4±12.29)of diabetic patients with insulin resistance did not induce any significant change** in level of serum Total cholesterol when compared with control group (gp.1),While the mean **± SD (145.5±33.57)**of HCV-infected group showed a significant decrease in the level of serum Total cholesterol when compared with group (2), on the contrary, the mean **± SD (182.7±14.31)of** increased tumor necrosis factor level did not reveal any significant change in the level of total cholesterol when compared with group (2),while the mean **± SD (222.1±24.681)** of coronary heart disease –group indicated a very highly significant elevation in the level of serum total cholesterol when compared with group(2).

The mean **± SD (51±4.77)of diabetic patients with insulin resistance did not induce any significant change** in level of serum HDL-cholesterol when compared with control group (gp.1),the mean **± SD (37.6±5.74)of** HCV-infected- group showed a very highly significant decline in the level of serum HDL-cholesterol when compared to group (2), while the mean **± SD (45.8± 3.39)** of the increased level of tumor necrosis factor-group revealed a significant decline when compared with group (2), on the contrary, the mean **± SD (37.6± 3.134)** of the increased level of tumor necrosis factor-group revealed a very highly significant decline when compared with group (2)also, the mean **± SD (37.6± 3.134)** of coronary heart disease-group showed a very highly significant decline when compared with group (2).

The mean **± SD (111.2±8.27)of diabetic patients with insulin resistance did not induce any significant change** in level of serum LDL-cholesterol when compared with control group (gp1), on the contrary, the mean **± SD (89.6±38.41)** of HCV-infected group did not induce any significant change in the level of serum LDL-Cholesterol when compared with group (2),also, the mean **± SD (118.3±11.99)** of increased level of tumor necrosis factor-group did not induce any significant any significant change in the level of serum LDL-Cholesterol when compared with group (2),on the contrary ,the mean **± SD (111.2±8.27)** of coronary heart disease-group revealed a very highly significant increase when compared with group (2).

The mean **± SD (134.9±19.68)of diabetic patients with insulin** resistance showed a highly significant elevation in the level of serum triglycerides when compared with control group (1),while the mean **± SD (98.4±30.53)** of serum triglyceride of HCV-infected group showed a very highly significant decline in the levels of serum triglycerides when compared with group (2),while the mean **± SD (96.9±14.46)** of serum triglyceride of tumor necrosis factor increased level- group showed a highly significant decline in the level of serum triglycerides when compared with group (2).while the mean **± SD (219.1±40.31)** of serum triglyceride of coronary heart disease –group showed a very highly significant elevation when compared with group (2).

The mean **± SD (31.92±2.15)of diabetic patients with insulin** resistance showed a very highly significant decline in the level of serum reduced glutathione when compared with control group (1),also the mean **± SD (17.13±3.20) of** HCV-infected group showed a very highly significant decline in the level of serum reduced glutathione when compared with group (2) and the mean **± SD (20.18±1.53)of** increased level of tumor necrosis factor group showed a very highly significant decline in the level of serum reduced glutathione when compared with group (2)and finally also the mean **± SD (29.36±1.591) of** coronary heart disease group showed a very highly significant decline in the level of serum reduced glutathione when compared with group (2).

The mean **± SD (9.01±0.96)of diabetic patients with insulin** resistance showed a very highly significant increase in the level of serum tumor necrosis factor when compared with control group (1),while the mean **± SD (93.9±2.73) of HCV-infected** group showed a significant increase in the level of serum tumor necrosis factor when compared with group (2) and the mean **± SD (9.87±0.81)of** increased level of tumor necrosis factor group showed a significant increase in the level of serum tumor necrosis factor when compared with group (2)and finally also the mean **± SD (8.04±1.04)of** coronary heart disease group showed a very highly significant decline in the level of serum tumor necrosis factor when compared with group (2).

There were also10 patients who had chronic hepatitis C virus(**gp.3**) that represents a 5% of the diabetic patients. Most of them had moderate degree of hepatitis, which was showed by the mean of alanine amino transferase level around three times upper normal limit 78.9±27.67 (u/l). Virological status showed that most of our hepatic patients had high viral load.

The effect of risk factors on the expression of CYP2E1 was carried out using RNA prepared from human peripheral blood and RT-PCR as shown in Table 2.The expression of **CRES-CYP2E1, CYP2E1** was positive in patients with DM with IR and HCV on the contrary, it was negative in diabetic patients without IR and HCV. The percentage of expression of **CRES-CYP2E1** was 80% while **CYP2E1** 40%.

Table (1): Means and Standard error of some biochemical parameters of the studied patients

Groups / parameters	Control DM without IR (A)	DM with IR (B)	DM with IR& HCV (C)	DM with IR&TNF-α (D)	DM with IR& CHD (E)
Age (years old)	57.7±4.6	51.7±6.1	52.6±3.5	54± 7.3	54.4±7.1
BMI (kg/m2)	28.04 ± 1.22	30.17±1.55	31.56±1.11	31.60±096	31.78±1.45
F-glu. (mg/dl)	123.6±12.77	207.5±36.23	191.2±13.67	191.5±39.47	232.7±45.4
Insulin	7.8±0.92	29±1.05	28.7±3.47	15±3.86	49.5±6.4
HOMA (%)	2.4±0.22	14.9±2.86	13.59±2.38	7.23±1.89	28.4±6.7
Alb. (g/dl)	3.9±0.35	3.92±0.26	3.02±0.19	3.18±0.25	3.96±0.22
ALT (u/l)	23.2±2.39	23.5±2.22	78.9±27.67	22.8±6.01	27.8±5.3
AST (u/l)	22.6±2.4	22.2±1.7	92.7±36.7	25.6±7.6	21±3.5
BUN (mg/dl)	13.3±2.01	12.9±1.66	18±2.78	13.2±2.1	17.6±2.47
Creat. (mg/dl)	0.86±0.22	0.87±0.15	0.96±0.35	0.8±0.17	1.04±0.18
T-chol. (mg/dl)	169.7±6.2	167.4±12.29	145.5±33.57	182.7±14.31	222.1±24.68
HDL-chol. (mg/dl)	50.7±4.16	51±4.77	37.6±5.74	45.8±-3.39	37.6±3.13
LDL-chol. (mg/dl)	105.5±8.20	111.2±8.27	89.6±38.41	118.3±11.99	164±17.81
Trig. (mg/dl)	113.6±7.24	134.9±19.68	98.4±30.35	96.9±14.46	219.1±40.31
Red.Glut. (mg/dl)	36.5±2.66	31.92±2.15	17.13±3.20	20.18±1.53	29.36±1.59
TNF-α	5.21±1.09	9.01±0.93	93.9±7.44	9.78±0.66	8.04±1.08

Table (2): The Percentage of expression of CRES-CYP2E1, CYP2E1 in patients with DM with and without HCV.

Groups / Parameters	Control DM without IR (A)	DM with IR& HCV (C)
Cres-cyp2e1	-ve .	80% +ve
Cyp2e1	- ve.	40% +ve.
HCV-PCR(Iu/ml)	-ve.	563200±712629.5

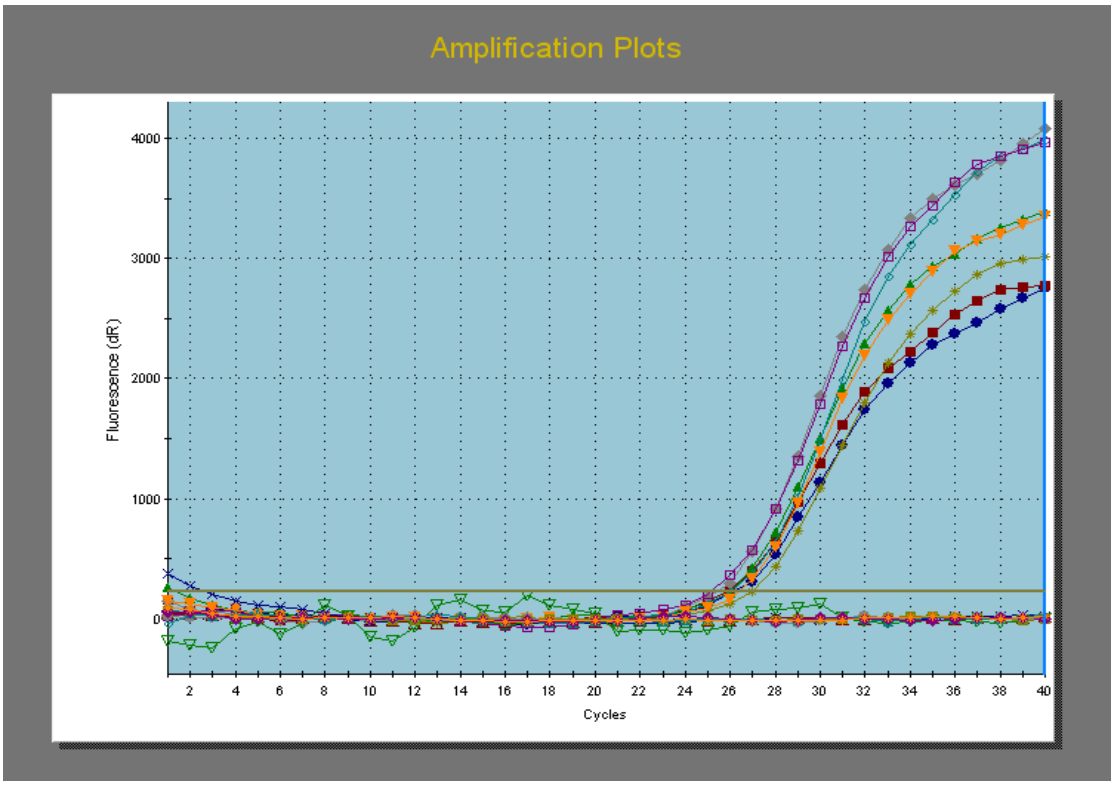

Figure (1): PCR instrument amplification plots (top panel) of Reactions patients samples.

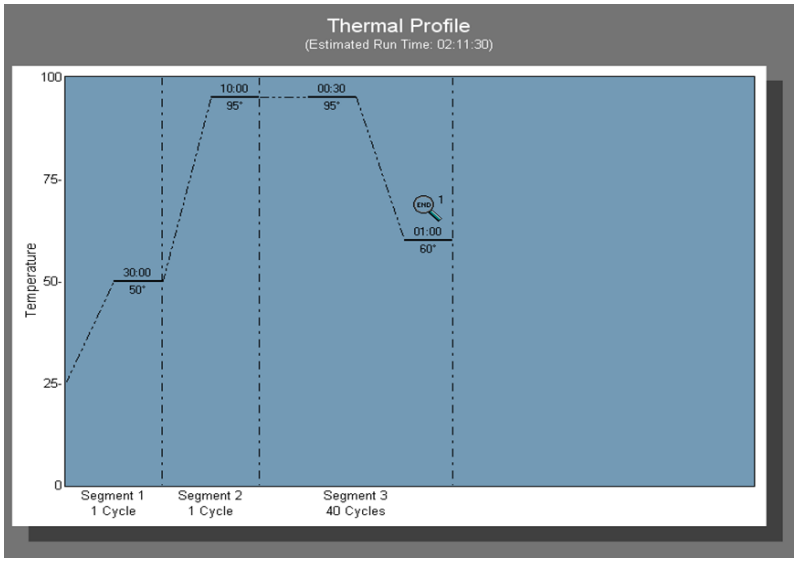

Figure (2): The thermal profile of the reaction.

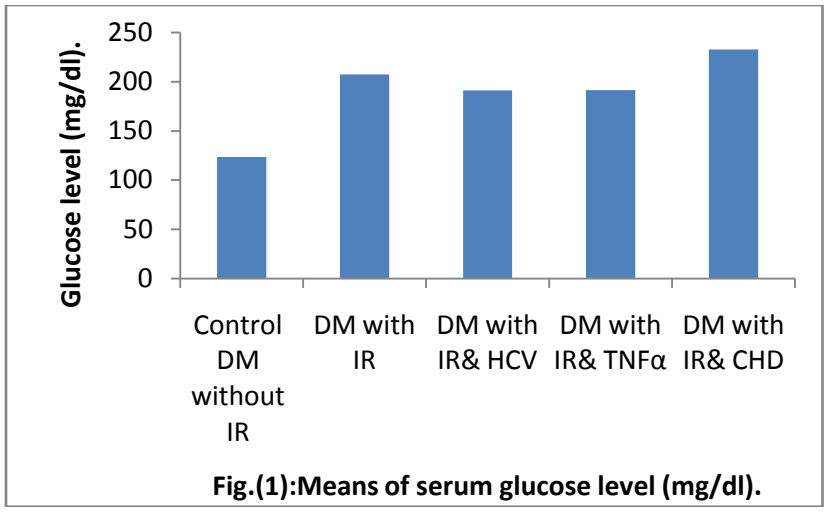

Fig.(1):Means of serum glucose level (mg/dl).

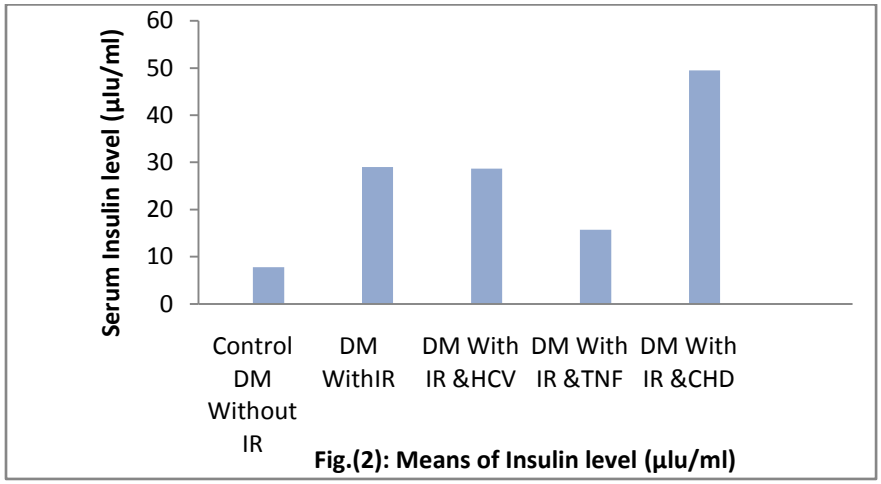

Fig.(2): Means of Insulin level (µlu/ml)

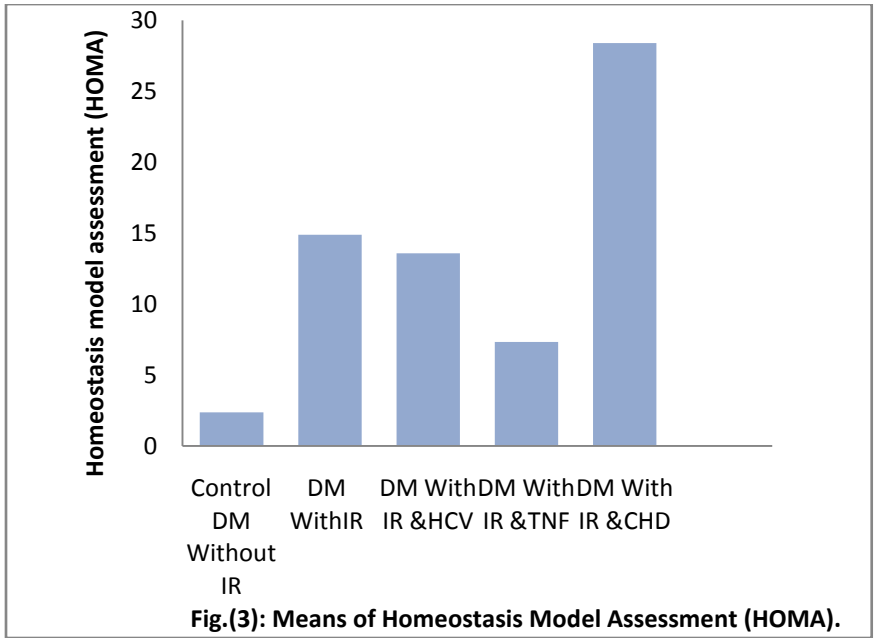

Fig.(3): Means of Homeostasis Model Assessment (HOMA).

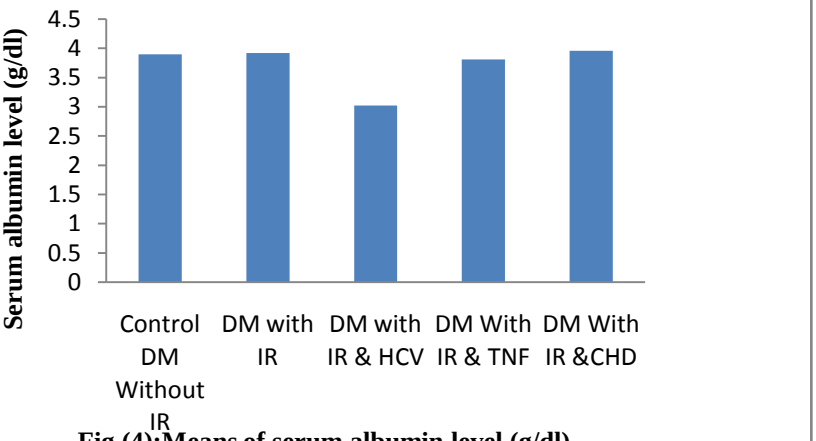

Fig.(4):Means of serum albumin level (g/dl).

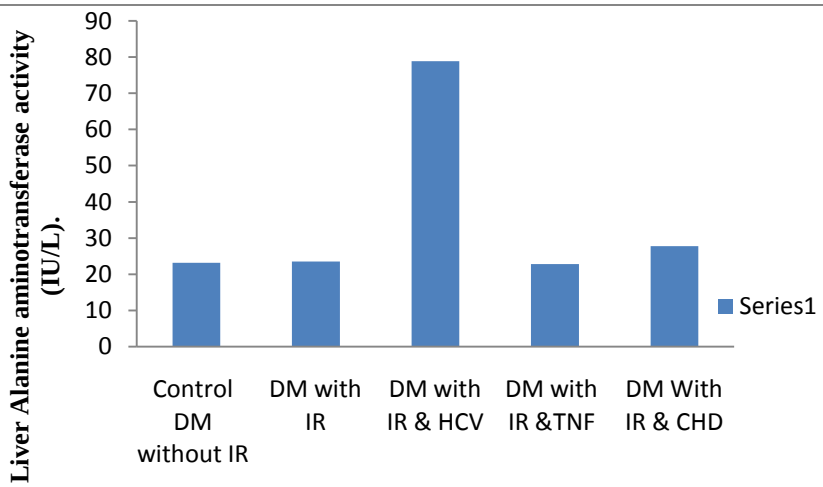

Fig.(5):Means of Liver Alanine aminotransferase activity (IU/L).

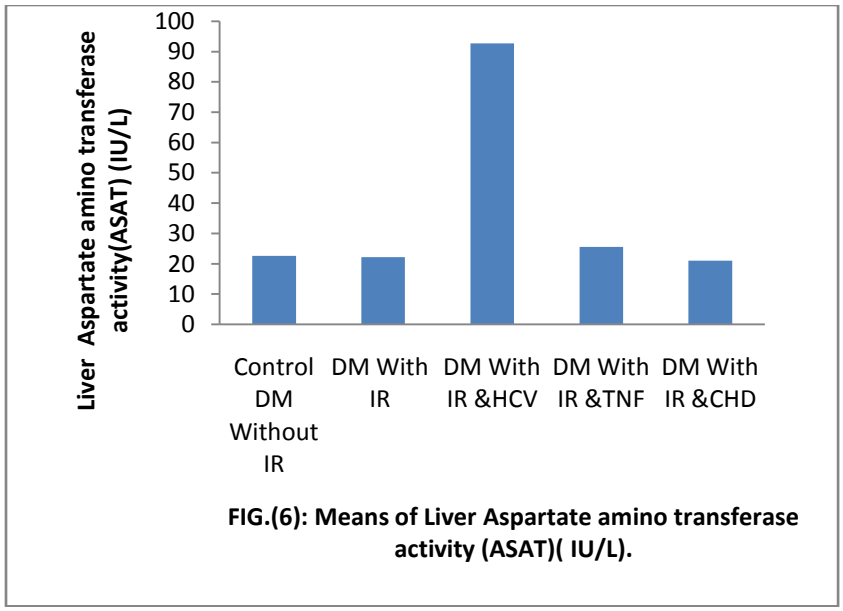

FIG.(6): Means of Liver Aspartate amino transferase activity (ASAT)(IU/L).

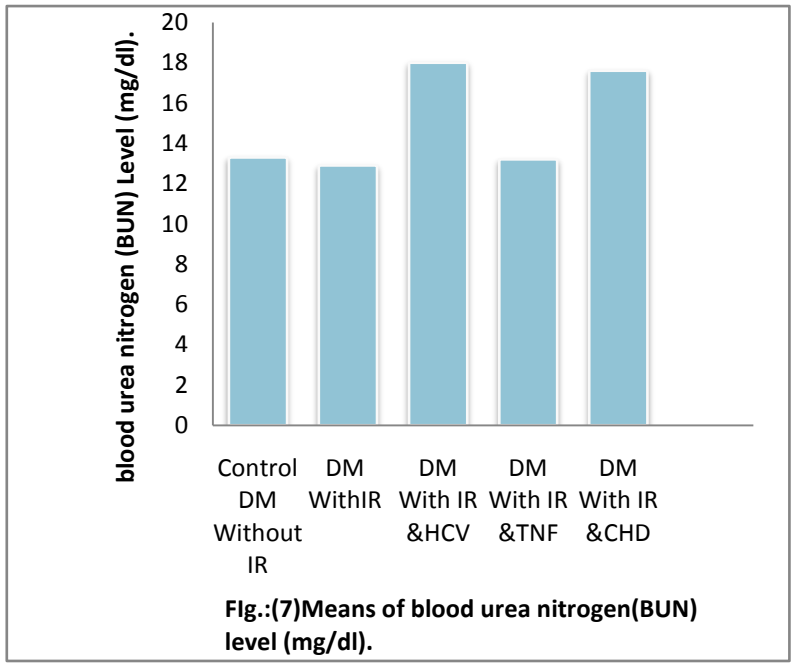

FIg.:(7)Means of blood urea nitrogen(BUN) level (mg/dl).

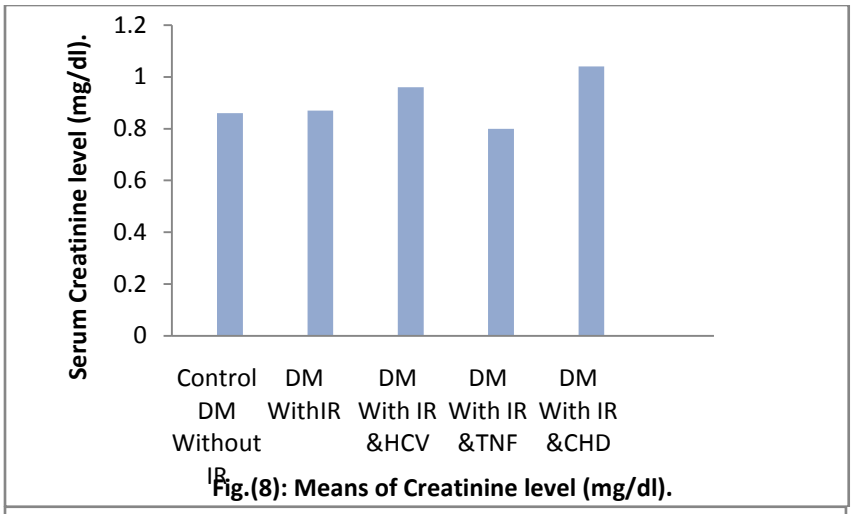

Fig.(8): Means of Creatinine level (mg/dl).

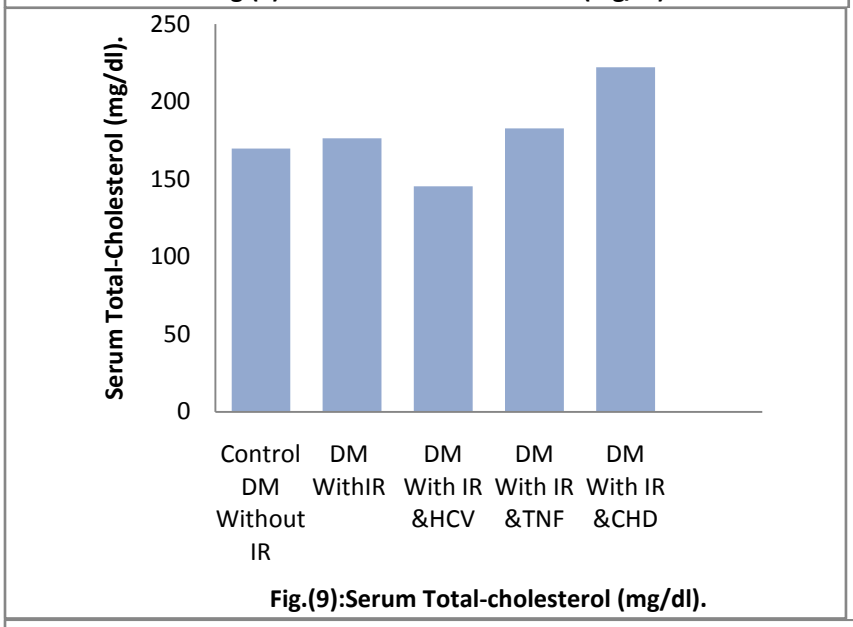

Fig.(9):Serum Total-cholesterol (mg/dl).

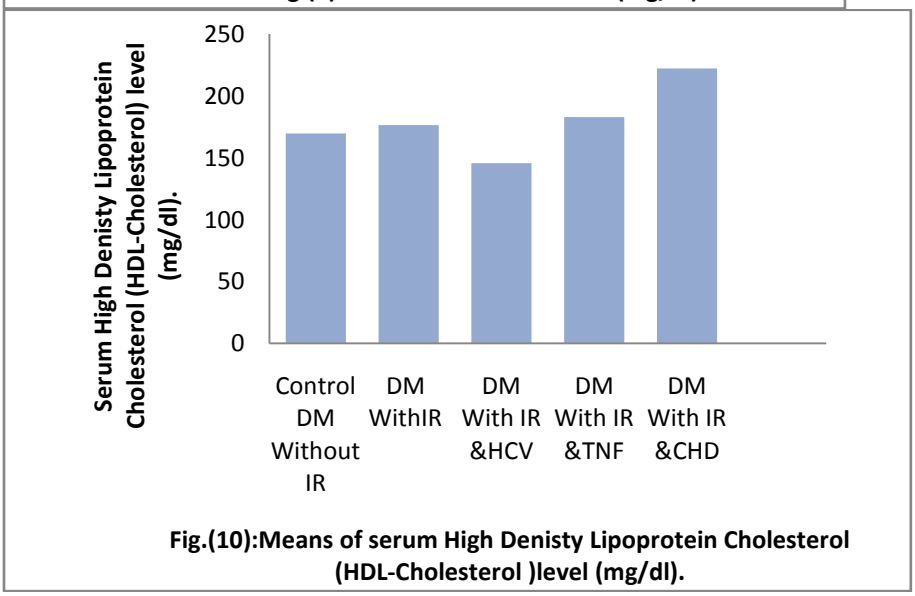

Fig.(10):Means of serum High Denisty Lipoprotein Cholesterol (HDL-Cholesterol)level (mg/dl).

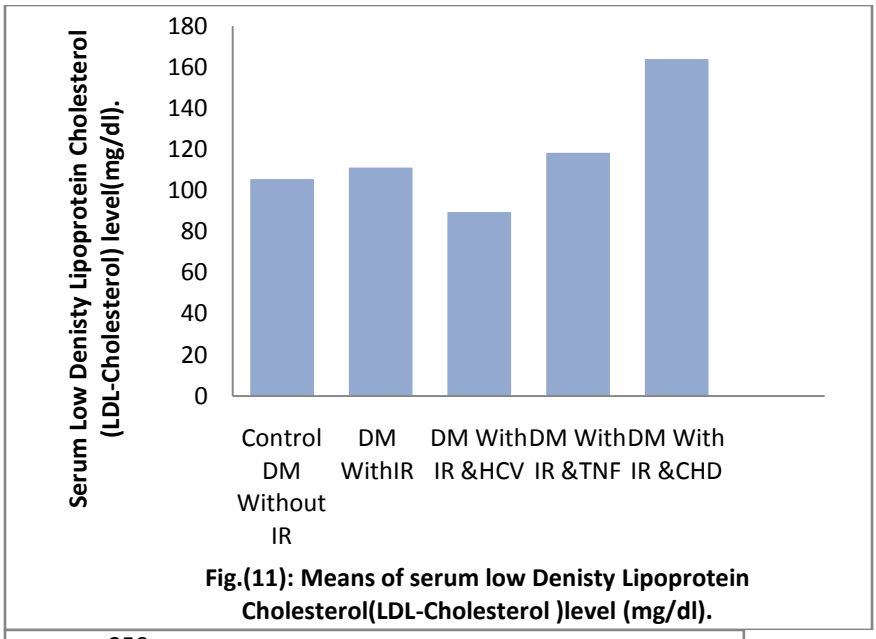

Fig.(11): Means of serum low Denisty Lipoprotein Cholesterol(LDL-Cholesterol)level (mg/dl).

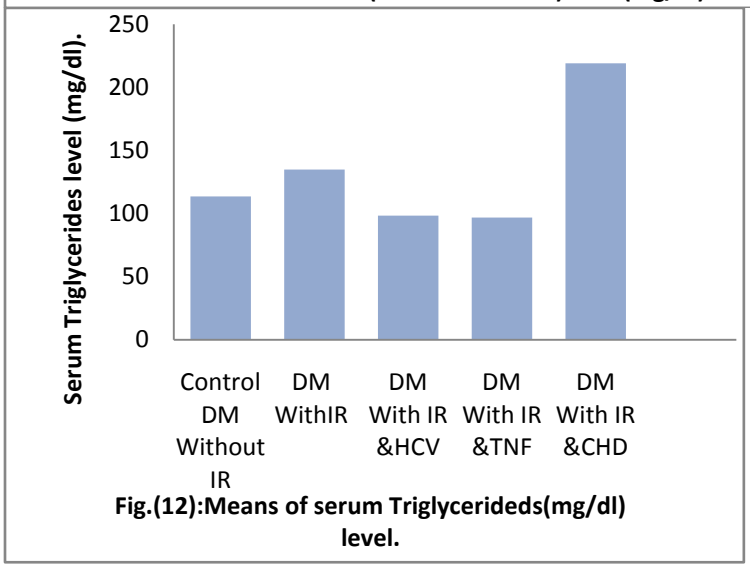

Fig.(12):Means of serum Triglycerideds(mg/dl) level.

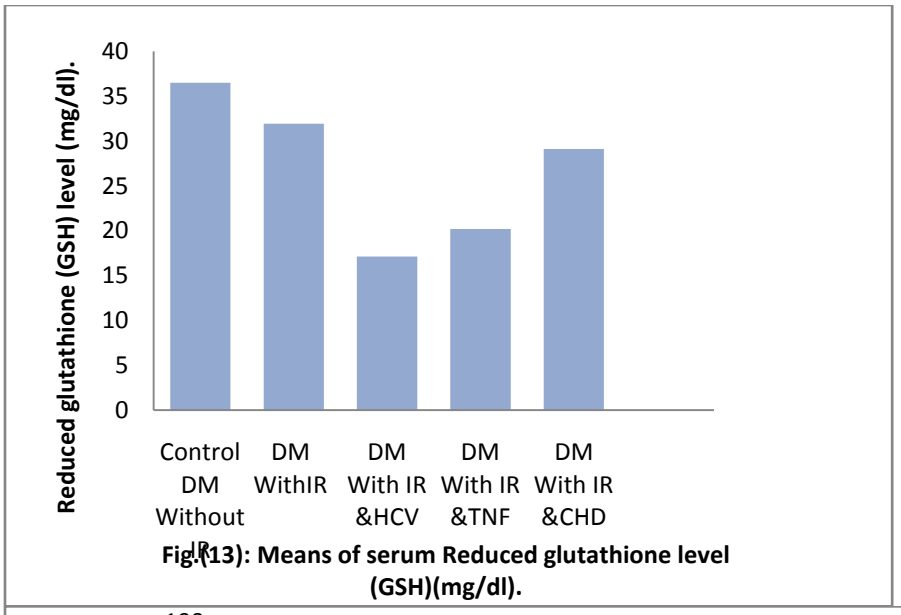

Fig.(13): Means of serum Reduced glutathione level (GSH)(mg/dl).

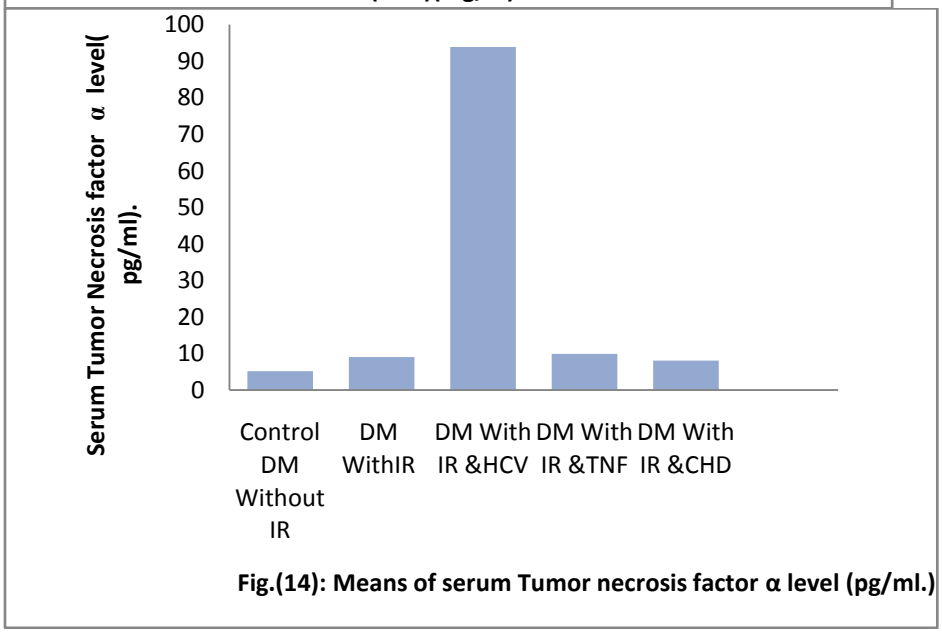

Fig.(14): Means of serum Tumor necrosis factor α level (pg/ml.)

Discussion:

Type 2 diabetes isn't just for adults anymore. The number of children and adolescents with the condition (most of whom are diagnosed in their early teens) has skyrocketed within the last 20 years, experts estimate that type 2 diabetes has grown from less than 5 percent in 1994 to about 20 percent of all newly diagnosed cases of the disease among youth in more recent years **(Chris and M.S., 2015).**Type 2 DM begins with insulin resistance, a condition in which cells fail to respond to insulin properly.As the disease progresses a lack of insulin may also develop (**Shi, Yuankai and Hu, 2012)**.IR is defined as a decreased biological response to normal concentrations of serum insulin that over time leads to compensatory hyperinsulinemia **(Kohen-Avramoglu , Theriault and Adeli , 2003).**

The following study showed that in group (**5**) the presence of coronary heart disease in the diabetic patients is significantly affected ($P<0.01$) by the body mass index in comparison to control group (**1**).This can be explained by the fact that obesity increases the risk of cardiovascular disease in adults and has been strongly associated with insulin resistance in individuals with type 2 diabetes; this result is in agreement with that of (**Cefalu *et al.*, 2015).**These abnormalities occur in many patients despite normal LDL cholesterol levels.

The change in the lipid profile is also a feature of the insulin resistance syndrome (also known as the metabolic syndrome), which underlies many cases of type 2 diabetes. In fact, pre-diabetic individuals often exhibit an atherogenic pattern of risk factors that includes higher levels of total cholesterol, LDL-cholesterol, and triglycerides and lower levels of HDL cholesterol than individuals who do not develop diabetes **(Haffner *et al*.,2000).**

Insulin resistance has striking effects on lipoprotein size and subclass particle concentrations for VLDL, LDL, and HDL (**Garvey et al ., 2003**).There is evidence that each of these dyslipidemia features is associated with increased risk of cardiovascular disease, the leading cause of death in patients with type 2 diabetes.

There was increasing evidence that HCV infection increases the risk for type 2 diabetes (DM) and insulin resistance (IR). In fact, previous study indicated that individuals older than 39 years of age and HCV infection increase the risk of DM by almost 4 times **(Younossi*et al*., 2013).** Similar studies showed the prevalence of DM increased according to the progression of liver disease in patients with HCV infection **(Harpreet et al., 2015).**

This study showed a higher prevalence of DM and IR in patients with HCV infection. However, other factors such as age, gender and BMI seemed to be the most important risk factors for the development of glucose abnormalities. The present study showed the prevalence of IR to be higher in patients infected with HCV (gp. **3**)($P<0.01$) than the other groups, These data suggest that patients with HCV are 3 times more likely to develop DM than individuals who are HCV negative. Therefore, Type 2DM is considered an extrahepatic manifestation of this infection.(**Juliana *et al*., 2012**).

The present investigation also showed that the activation of the tumor necrosis factor (TNF-α) in the diabetic HCV patients in (gp.**3**)($P<0.01$) in compared to the control group (**1**).TNF-α has a pivotal role in the inflammatory process of chronic hepatitis C, and the TNF-α levels correlate with the degree of inflammation. TNF-α is known to cause insulin resistance, with similar defects in the insulin signaling pathway to those described in HCV infection this is with agreement with the study of A model of mice transgenic for the HCV core protein demonstrated insulin resistance, glucose intolerance, and elevated intrahepatic TNF-α mRNA; all of which were ameliorated by anti-TNF-α antibodies. In addition, diabetic HCV patients have significantly higher levels of soluble TNF-α receptors, compared to non-diabetic HCV patients and controls. TNF-α may be the link between HCV infection and diabetes, suggesting an additional mechanism of diabetes with important implications for prognosis and therapy **(Knobler and Schattner, 2005)**.

The diagnosis of acute hepatitis C virus (HCV) infection is infrequently made, primarily because more than 70% of patients do not have symptoms associated with the acute infection Overall, approximately 25% of all patients with acute HCV present with jaundice, and 10 to 20% develop gastrointestinal symptoms (nausea, vomiting, or abdominal pain)(**Stephen ,2006**). As would be expected, amongthose persons with clinically recognized acute HCV, the reported rates of symptoms are much higher and typically include jaundice, fatigue, flu-like symptoms, and dark urine (**Gerlach*et al*., 2003**). In many cases, laboratory abnormalities may provide the initial clue to suggest a diagnosis of acute HCV infection, rising alanine amino transferase (ALAT) activity are typically observed approximately 40 to 50 days after infection (**Glynn *et al*., 2005**).the present study showed that Patients were significantly older and had higher serum ASAT and ALAT activity ($P<0.01$) and Serum albumin levels were highly significantly lower ($P<0.01$) in patients with HCV (gp.3) than in those without HCV infection.

In the following study the presence of hepatitis c virus in the diabetic patients (gp. **3**) make a highly significant increase ($P<0.01$) in BUN level in compared to control group (1) and doesn't make a significant change ($P>0.05$) in creatinine level in compared to control group (1).on the contrary **Wang *et al.* (2010)** reported that BUN and creatinine were not significantly different between patients with HCV infection and those without.

GSH plays a crucial role in the protection of cells against a diversity of injuries, and GSH depletion leads to increased susceptibility of the cells to the harmful effects of free radicals and toxic metabolites and may be a factor involved in cell lysis. In addition, since intracellular GSH levels influence the activity of nuclear factors, reduced levels of the thiol may alter gene expression and viral replication. In the present investigation, the measured levels of GSH in serum of all groups including patients with chronic HCV revealed a highly significant reduced ($P<0.01$)

level of GSH in HCV group (gp. **3**) Than other groups these results are in agreement with those of**(Czeczot,2006)** that attributed this depletion to liver disease.

Human cytochromes P-450 (CYP) are a superfamily of mixed function mono-oxygenases encoded by at least 50 different genes grouped in 10 families and sharing approximately 40% sequence homology. CYPs are mainly expressed in the liver; the CYP1, CYP2 and CYP3 family genes are located in the endoplasmic reticulum, and their major role is the metabolism of xenobiotics. CYPs are responsible for phase I enzyme reactions, resulting in the creation of functional groups and "reactive centers" on substrates (e.g., -OH, -NH2, -SH and -COOH), which prepare them for phase II conjugation reactions, but they can also lead to the metabolic activation of toxic or carcinogenic compounds (**Silvestri *et al*., 2003).**

There is an increase in prevalence of hepatic steatosis in chronic HCV. There were reports that hepatic steatosis impaired response of chronic hepatitis C treatment and developed more fibrosis. Insulin resistance was reported to be associated with hepatic fibrosis. Cytochrome P450 2E1 also had shown some associations with steatosis of liver tissues (**Kalsi*et al*., 2011)**.

The pathogenesis of steatosis in chronic viral hepatitis is poorly understood. In chronic hepatitis C, there were many mechanisms proposed in several literatures. Steatosis may occur based on viral and host factors. There was evidence that insulin resistance acted as the "first hit" of pathogenesis of Steatohepatitis then followed by the second hit, the oxidative stress. Oxidative stress marker that we studied was cytochrome P450 2E1activity. There was a study that showed an over expression of CYP 2E1 in the liver of chronic hepatitis C patients with steatosis compared with those without steatosis.(**Craig*et al* .,2009**) Some data suggested that CYP 2E1 overexpression down regulated insulin signaling and contributed to insulin resistance (**Jiangzheng *et al* .,2015**), these results are line with results of the following experiment.

From the present study, 40 % of HCV patients were over-expressed for cytochrome P2E1 (CYP 2E1) and 80 % for Crs- cytochrome P2E1 (Cres-CYP2E1. Most of our patients had moderate degree of hepatitis which was showed by the mean of alanine amino transferase level around three times upper normal limit. Virological status showed that most of our patients had high viral load. Insulin resistance was postulated to be the cause of hepatic steatosis. One study showed that the higher the degree of insulin resistance, the lower the sustained virological response rate (**D'Souza*et al*., 2005**). Moreover, other studies showed that insulin resistance played a significant role in liver fibrosis (**Romero-Gomez*et al*., 2005 and Mohammad*et al*., 2006).**Interestingly, those studies did not show the risk of metabolic syndrome despite their studied patients had higher mean body mass index and insulin resistance. Our study results showed higher HCV load with increase in IR and BMI.Cytochrome P450 2E1 activity is a representative of oxidative stress as a "second hit" in the pathogenesis of HCV. From the previous, CYP 2E1 overexpression was more commonly found in liver tissue from chronic hepatitis C patients. The cytochrome P450 2E1 activity was also reported to be greater in the liver tissue of diabetic patients with HCV than in normal.

Reference:

Atègbo, M. (2006). Modulation of adipokines and cytokines in gestational diabetes and macrosomia.J. Clin.Endocrinol.Metab.,**91**(10):4137-4143.

Boussageon, R.; Bejan-Angoulvant, T. and Saadatian-Elahi, M. (2011). Effect of intensive glucose lowering treatment on all causes of mortality, cardiovascular death, and microvascular events in type 2 diabetes: meta-analysis of randomisedcontrolled trials.B.M.J., **343**: 4169-4175.

Cefalu, W.T.; Bray, G.A.; Home, P.D.; Garvey, W.T.; Klein, S.; Pi-Sunyer, F.X.; Hu, F.B.; Raz, I.; Van Gaal, L.; Wolfe, B.M. and Ryan, H.D.(2015). Advances in the Science, Treatment, and Prevention of the Disease of Obesity: Reflections From a Diabetes Care Editors' Expert Forum.Diabetes Care.,**38**(8):1567-1582.

Chehadeh, W.; Abdella, N.; Ben-Nakhi, A.; Al-Arouj, M. and Al-Nakib, W. (2009).Risk factors for the development of diabetes mellitus in chronic hepatitis C virus genotype 4 infection.J. Gastroenterol.Hepatol., **24**(1):42-48**.**

Chris Woolston, M.S.(2015).Diabetes Health Library .Lime Health.

Craig, D. F.; Andrew, J. L.; Lisa, M. A.; James, R.; Jonathan, P. J.; Stephen, S. F. and Nathan J. C. (2009). Hepatic Cytochrome P450 Enzyme Alterations in Humans with Progressive Stages of Nonalcoholic Fatty Liver Disease Drug Metab.Dispos., **37**(10): 2087–2094.

Czeczot, M.; Ścibior, D.; Skrzycki, M. and Podsiad, M.(2006). Glutathione and GSH-dependent enzymes in patients with liver cirrhosis and hepatocellular carcinoma Acta. Biochemica . (**53**) **1**: 237–241.

D'Souza, R.; Sabin, A. and Foster, G.R. (**2005)** Insulin resistance plays a significant role in liver fibrosis in chronic hepatitis C and response to antiviral therapy. A.m. J. Gastroenterol., **100**:1500- 1509.

Enzo, B.; Gianni, F. and Francesco, C. (2002). HOMA-Estimated Insulin Resistance Is an Independent Predict of Cardiovascular Disease in Type 2 Diabetic Subjects .Diabetes Care, **25**:1135–1141.

Fox, C.S.;Golden, S.H.; Anderson, C.; Bray, G.A.;Burke, L.E.; Boer, I.H.; Deedwania, P.; Eckel, R.H.; Ershow, A.G.; Fradkin, J.; Inzucchi, S.E. and Kosiborod, M.(2015). Update on Prevention of Cardiovascular Disease in Adults With Type 2 Diabetes Mellitus in Light of Recent Evidence: A Scientific Statement From the American Heart Association and the American Diabetes Association. **38**(9):1777-1803.

Garvey, W.T.; Kwon, S.; Zheng, D.; Shaugh-nessy, S.; Wallace, P.; Hutto, A.; Pugh, K.; Jenkins, A.J.; Klein, R.L. and Liao, Y.(2003). Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance.Diabetes **.,52**:453–462.
Gerlach, J.T.; Diepolder, H.M. and Zachoval, R. (2003). Acute hepatitis C: high rate of both spontaneous and treatment-induced viral clearance. Gastroenterology. **125:80**-88.

Glynn, S.A., Wright, D.J. and Kleinman, S.H.(2005).Dynamics of viremia in early hepatitis C virus infection.Transfusion ., **45**:994-1002.

Gochee, P.A.; Jonsson, J.R.; Clouston, A.D.; Pandeya, N.; Purdie, D.M. and Powell, E.E. (2003).Steatosisin chronic hepatitis C: association with increased RNA expression of collagen I, tumor necrosis factor-α and cytochrome P4502E1. J. Gastroenterol.Hepatol**., 18:**386–392.

Haffner, S.M.; Mykkanen, L.; Festa, A.; Burke, J.P. and Stern, M.P. (2000). Insulin-resistant predia-betic subjects have more atherogenic risk factors than insulin-sensitive predi-abetic subjects: implications for preventing coronary heart disease during the prediabeticstate.Circulation**, 101:975**–980.
Harpreet,K.;Parminder, S.; Harminder, S. P.; AjitSood, N. P. and Harpreet S. B.(2015). To Study the Prevalence of Impaired Glucose Tolerance in Patients with Hepatitis C Virus Related Chronic Liver Disease. J.Clin.Diagn. Res., **9**(3):16–20.

Jiangzheng, L.; Xin, W.; Zhengwu, P.; Tao, Z.; Hao, W.; Weihua, Y., Deqing, K.; Ying, L.; Hua, B.; Rui, L.; Xiaodi, Z. and Chunxu, H. (2015). The effects of Insulin Pre-Administration in Mice Exposed to Ethanol: Alleviating Hepatic Oxidative Injury through Anti-Oxidative, Anti-Apoptotic Activities and Deteriorating Hepatic Steatosis through SRBEP-1c Activation.Int. J. Biol. Sci., **11**(5): 569–586.

Juliana, C.; Janine, C. and Cresio, A.(2012). Infections in patients with diabetes mellitus: A review of pathogenesis. Indian J. Endocrinol.Metab., **16**(1): 27–36.

Kalsi, S.S.; Wood, D.M.; Waring, W.S. and Dargan, P.I.(2011) . Does cytochrome p450 liver isoenzyme induction increase the risk of liver toxicity after paracetamol overdose? Open Access Emerg. Med.**, 3:**1–8.
Knobler, H. and Schattner, A.(2003).Clinical aspects of chronic hepatitis C infection. J.A.M.A., **290**(11): 1453-1454.

Kohen-Avramoglu , R.; Theriault, A. and Adeli, K. (2003). Emergence of the metabolic syndrome in childhood: An epidemiological overview and mechanistic link to dyslipidemia. Clin.Biochem.**36**:413-420.

Kuiken, C.; Yusim, K.; Boykin, L. and Richardson, R.(**2005).**The Los Alamos HCV Sequence Database. Bioinfomatics **.,21(**3):379-384.
Levent, G.; Ali, A.; Ahmet, A.; Polat, E.C.; Aytac, C.; Ayse, E. and Ahmet, S. (2006). Oxidative stress and antioxidant defense in patients with chronic hepatitis C patients before and after pegylated interferon alfa-2b plus ribavirin therapy. J. Transl.Med **., 4:**25–30.

Loaeza-del Castillo, A.(2005).Association between diabetes, overweight, obesity and dyslipidemia with fibrosis progression in chronic hepatitis C patients. 40th E.A.S.L. Paris, France **581**.

Mastoi, A.A.; Devrajani, B.R.; Shah, S.Z.; Rohopoto, Q.; Memon, S.A.; Baloch, M.; Qureshi, G.A. and Sami, W.(2010). Metabolic investigations in patients with hepatitis B and C. World J. Gastroenterol., **16**(5):603-607.

Mohammad, A.A.H., Fallahian, F. and Alavian, S.M.(2006). Insulin resistance in chronic hepatitis B and C. Indian J. Gastroenterol., **25**: 286-289.

Nelson, D.R.; Zeldin, D.C.; Hoffman, S.M.; Maltais, L.J.; Wain, H.M. and Nebert, D.W. (2004). Comparison of cytochrome P450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice variants. Pharmacogenetics**,14**:1–18.

Romero-Gomez, M.; Del Mar, V.M. and Andrade, R.J.(2005). Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. Gastroenterology. **128**: 636-641.

Shi, N.; Yuankai, L. and Hu, F.B. (2012). Textbook of diabetes mellitus. New Delhi: Jaypee Brothers Medical Publishers. , **2**: 235-240.

Shlomo, M.; Kenneth, S.; Larsen, P. and Henry, M.(2011).Williams textbook of endocrinology . Philadelphia: Elsevier/Saunders. **12**: 1371–1435.

Silvestri, L.; Sonzogni, L.; De Silvestri, A. and Gritti, C. (2003). CYP enzyme polymorphisms and susceptibility to HCV-related chronic liver disease and liver cancer. Int. J. Cancer **104**: 310-317.

Sizmann, D.; Boeck, C. and Boelter, J.(2007). Fully automated quantification of hepatitis C virus(HCV) RNA in human plasma and human serum by the COBAS AmpliPrep/COBAS TaqMan system. J. Clin. Virol.,**38**:326-333.

Smith, E.S.; Li, A.K.; Wang, A.M.; Gelfand, D.H. and Myers, T.M. (2003). Amplification of RNA:High-Temperature Reverse Transcription and DNA Amplification with a Magnesium-Activated Thermostable DNA Polymerase. In PCR Primer: A Laboratory Manual, Dieffenbach C.W. and Dveksler G.S., Eds. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, **2**: 211-219.

Stephen, L. C.; Timothy, R. and Morgan. (2006). The Natural History of Hepatitis C Virus (HCV) Infection. Int. J. Med. S.c.i., **3**(2):47-52.

Swardfager, W.; Lanctôt, K.; Rothenburg, L.; Wong, A.; Cappell, J. and Herrmann, N. (2010). A meta-analysis of cytokines in Alzheimer's disease. Biol. Psychiatry, **68** (10): 930–941.

Tabish,S.A.(2007). Is Diabetes Becoming the Biggest Epidemic of the Twenty-first Century? :Int. J. Health S.c.i. (Qassim).,**1**(2): 5–8.

Toth, P. (2005). Cardiology patient page. The "good cholesterol": high-density lipoprotein. Circulation **,111** (5): 89–91.

Turner,J.R.(2001). Introduction to analysis of variance: Design, analysis & interpretation. sage publication. , thousand oaks, C.A.

Wang, S.M.; Liu J. H.; Chou, C.Y.; Huang, C. C. ; Shih, C. M. and Chen, W.**(2010**)Division of Nephrology,1 Department of Internal Medicine, China Medical University Hospital,Taichung; Division of Nephrology,2 Department of Internal Medicine,China Medical University Beigang Hospital, Yunlin, Taiwan .Peritoneal Dialysis International., **28**: 183–187.

Younossi ,Z.M. M.: Stepanova, F. N.; Younossi,Z. and Elsheikh,E. (2013). Associations of chronic hepatitis C with metabolic and cardiac Outcomes.Center for Liver Diseases,Department of Medicine, Inova Fairfax Hospital, Falls Church, V.A., U.S.A.,**37**(6):1-6.