Volume No. 48: (1&2)

January & July 2016

The Journal of the Egyptian Society of Endocrinology, Metabolism & Diabetes

Journal Of The Egyptian Society of Endocrinology, Metabolism & Diabetes.

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Limit the abstract to 250 words. Use a structured format, including Aim, Subjects and Methods, Results, and Conclusions. Provide 3-6 key words for indexing at the end of the abstract. Provide a list of abbreviations used throughout the manuscript, arranged alphabetically, at the bottom of the first page.

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Journal

1. **Van den Berghe G, Wouters P, Weekers F, et al.** Intensive insulin therapy in critically ill patients. *N Engl J Med* 2001; 345:1359-1367.

Book

2. **Falk SA, ed.** *Thyroid Disease: Endocrinology, Surgery, Nuclear Medicine, and Radiotherapy.* 2nd ed. Philadelphia: Lippincott-Raven, 1997.

Chapter in Book

3. **Flier JS, Foster DW.** Eating disorders: obesity, anorexia nervosa, and bulimia nervosa. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR, eds. *Williams Textbook of Endocrinology*. 9th ed. Philadelphia: WB Saunders, 1998: 1061-1097.

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ISSN: 1110 – 1245 (print)
ISSN: 2535 – 1745 (On Line)

Journal Of The Egyptian Society of Endocrinology, Metabolism & Diabetes

Contents

Original Articles

Letter From The Editor

Dear Colleague,

In this volume, we have included a very important article which deals with a hot and debatable issue. This is “The Effect of the Tight Glycemic Control Coupled with the Use of a Continuous Glucose Monitoring System on the Outcome of Acute Myocardial Infarction. This work has been leaded by Prof. Paresh Dandona Team.

As promised we initiated a website for our journal, which is: www.jesemd.com

Once again, we hope to meet your expectations, and until we meet in our next issue, deepest regards and best wishes.

The Editor

Prof. Samir Helmy Assaad Khalil

ISSN:1110 - 1245 (print)
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Original Article

The Effect of the Tight Glycemic Control Coupled with the Use of a Continuous Glucose Monitoring System on the Outcome of Acute Myocardial Infarction.

Samir Assaad-Khalil [1], Paresh Dandona [2], Hesham El Ashmawi [3], Ajay Chaudhuri [2], Hassan Abu Khabar [4], Talaat Abd El Aty [1], Mohamed Megahed [1], Hany Assaad-Khalil [4].

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Abstract:

Objective: To investigate whether lowering blood glucose to <120mg/dl with an insulin infusion in STEMI will improve myocardial function, reduce hs-CRP and CK concentrations and lower the incidence of major adverse cardiac events (MACEs). We also investigated the effectiveness of continuous blood glucose monitoring in the setting of STEMI. **Methods:** 50 patients with STEMI receiving thrombolytic therapy within 6 hours of developing symptoms, were randomized into 3 groups: intensive insulin infusion therapy (IIT); glucose-insulin-potassium infusion (GIK), with both groups targeting a glucose of 80-120 mg/dl and a control group targeting glucose concentrations of 140-150 mg/dl. Blood glucose concentrations were monitored using a capillary blood glucometer 2 hourly or a continuous glucose monitoring (CGM) device. **Results:** The improvement of LVEF and WMSI in both IIT and GIK groups at 3 and 30 days after admission was greater than that in the control group. The hs-CRP and peak CK concentrations and the incidence of MACEs were significantly lower in IIT and GIK groups than those in the control group, ($p<0.001$, 0.05 & 0.015). The percentage of in-target glucose concentrations was significantly higher in the patients monitored using the CGM than that in patients having 2 hourly glucometer readings (56.85±12.04%, 82.92±5.77%, $p<0.001$). The incidence of hypoglycemia was significantly lower in patients monitored using CGM ($p = 0.007$). **Conclusions:** Tight glycemic control in ITT and GIK groups resulted in a significantly reduced peak CK and CRP concentrations, less myocardial dyskinesia, an improved ejection fraction and a lower incidence of MACE in patients with STEMI when compared to controls. CGM facilitated the attainment of tight glycemic control with a decreased incidence of hypoglycemia.

Keywords: Tight Glycemic Control – CGM- Acute Myocardial Infarction

Introduction:

Admission hyperglycemia (> 140mg/dl) in patients with acute myocardial infarction (AMI) is associated with increased morbidity and mortality.[1,2] while a decrease in glucose concentrations following admission have been shown to be associated with a reduction in mortality and morbidity.[3,4] Hyperglycemia exerts potent pro-inflammatory, pro-thrombotic and pro-apoptotic effects,[5-13] while an insulin infusion exerts potent anti-inflammatory, anti-platelet, pro-fibrinolytic and anti-apoptotic effects in addition to lowering blood glucose concentrations. [14-22]

In spite of the potentially beneficial effects of insulin in acute myocardial infarction (AMI), the outcomes from the insulin infusion trials have been variable. In most of these studies, a fixed dose of insulin was infused with a high dose of glucose and potassium [23, 24]. This often led to hyperglycemia and fluid overload.. Some trials have infused insulin in

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hyperglycemic patients to reduce glucose but never to <140mg/dl. [25-27]

We, therefore, investigated whether the restoration and maintenance of euglycemia (blood glucose target < 120mg/dl) with insulin either given alone or as a GIK infusion in patients with STEMI would exert a beneficial effect on myocardial function and on post-AMI increases in plasma CK and hsCRP concentrations when compared to patients with blood glucose concentrations between 140-150mg/dl. Furthermore, we investigated whether hypoglycemia could be avoided while maintaining blood glucose concentrations <120 mg/dl by using a continuous blood glucose monitoring system.

Patients:

The present study included 50 patients admitted to the Alexandria Main University Hospital from the 1st of June 2008 to January 2009 with the following criteria: Acute ST segment elevation myocardial infarction (STEMI) for the first time, diagnosed by symptoms consistent with AMI ≥ 20 minutes in duration, and new ST elevation at the J-point in two contiguous leads with the cut-off points: ≥0.2 mV in men or ≥0.15 mV in women in leads V2, V3 and/or ≥0.1 mV in other leads.[28] Killip class I-II, presenting within 6 hours of symptoms onset. An informed consent was obtained from all subjects or their first degree relatives.

Methods:

This is a prospective, randomized, controlled, open label study. All subjects received Streptokinase (1.500.000U+100 ml of 5% glucose) intravenously over one hour. They were also given aspirin, clopidogrel, a β-blocker, an ACE inhibitor and a statin, according to a protocol adopted by the Alexandria Main University Hospital (AMUH) consistent with the AHA/ACC guidelines. [29]

The patients were randomized into 3 groups:

Intensive insulin therapy (IIT) group: 20 patients received IIT in the form of continuous intravenous insulin infusion targeting normoglycemia (80-120 mg/dl) for 72 hrs post MI. The insulin infusion was initiated before thrombolytic therapy. We chose a target glucose of 80-120mg/dl since AMI patients with post admission glucose of 80 to 130mg/dl have the lowest mortality. [30]

Glucose-insulin-potassium (GIK) with Tight Glycemic control group: 15 patients received a high dose GIK infusion[31] for the first 24 hours of admission in addition to a continuous intravenous insulin infusion to maintain glucose between 80-120mg/dl for 72 hrs post MI. An Intravenous infusion of 1 liter 25% glucose + 40 U Insulin (ActRapid) + 80 meq Potassium, was started prior to thrombolytic therapy in a large peripheral or central vein,[32] at a rate of 1 ml /kg /hour. Additional intravenous insulin infusion was started, if required to maintain blood glucose between 80-120mg/dl. We used a high dose GIK infusion as a low dose (1U/h) GIK infusion has not been shown to be beneficial in AMI.

Control Group: 15 patients received subcutaneous doses of Regular insulin every 6 hrs to maintain glucose below 150 mg/dl for 72 hrs post MI, according to a sliding scale protocol. A glucose target of below 150mg/dl is in accordance with the American Heart Association's scientific statement on hyperglycemia and acute coronary syndrome. [33]

In all groups, the other anti-diabetic medications were withdrawn for 72 hrs in subjects with known diabetes

Insulin Infusion protocol in the IIT and GIK group to maintain tight glycemic control:

In the GIK and the IIT groups, blood glucose (BG) concentrations were maintained by following a protocol, modified from the LEUVEN Protocol[34]. 50 Unit Insulin (ActRapid) was added to 50 ml normal saline and infused by a syringe pump.

i. If BG was > 220 mg/dl on admission, insulin 2-4 U/h was started.

ii. If BG was 120-220 mg/dl on admission, insulin 1-2 U/hour was started.

iii. If BG was 80-119 mg dl on admission, insulin was initiated depending on the next RBG value one hour later.

iv. Insulin dose was adjusted according to the blood glucose values measured two hourly:

v. If BG was > 140 mg/dl, insulin was increased by 1-2 U/hour.

vi. If BG was 120-140 mg/dl, insulin dose was increased by 0.5-1 U/hour.

vii. If BG was 90-119 mg/dl, insulin was adjusted upon the next BG value after 2 hours.

viii. If BG fell between 60-89 mg/dl insulin was reduced by half and blood glucose was measured after 30 minutes after assuring adequate baseline glucose intake.

ix. If BG was <60 mg/dl, insulin was stopped, 10 g Glucose IV bolus was administered and BG was re-measured after 30 minutes.

In the Control group blood glucose target was maintained < 150mg/dl for 3 days post admission with the use of a sliding scale every 6 hours. (Appendix 1)

Monitoring of Glycemic Control:

All patients were randomized for their BG monitoring into:

1) BG was measured by a glucometer "AccuCheck / Go – by Roche" every 2 hours for 3 days.

2) RBG measured by continuous blood glucose level monitoring device "GUARDIAN System-by Medtronic / USA" for 3 days. [35]

For statistical comparison and to reduce bias of readings between the different groups, capillary testing was done in all patients, even those monitored by the Guardian system. Between scheduled capillary readings, if the Guardian system alerted the physician about potential hypoglycemia or out of target glucose, changes to caloric intake and/or insulin infusion rate were made, until the next capillary testing and confirmation. Physicians were allowed to confirm the Guardian readings by capillary testing at any time.

Serum CK was measured on admission, 90 minutes, 6 hours, 12 hours and then every 12 hours for 3 days. High sensitivity C - reactive protein (CRP) was measured on admission and at 48 hours.

To assess reperfusion success, myocardial function and cardiac complication the following were monitored:

a) ECG/ Symptoms/ CK: Resolution of ST elevation more than 50% on ECG performed within 90 minutes of reperfusion therapy/the absence of electrocardiographic Q waves. Chest pain resolved during or just after (within 90 minutes) of reperfusion therapy; Reperfusion arrhythmia during reperfusion therapy in the form of accelerated idioventricular rhythm (rate equal or below 100 b/min) or sinus bradycardia (below 55 beats/min) in inferior MI; Creatinine Kinase (CK) peak level. [36, 37]

b) A two dimension trans-thoracic Echo was done on admission, day 3 and at one month for: assessment of: Left ventricular ejection fraction using the Simpson's method and Wall motion score index calculated with the 16-segment model of the American Society of Echocardiography. [38]

In hospital and 30 day Major Adverse Cardiac Events (MACEs) were defined as a composite of:

1. Cardiac death defined as any death from cardiac causes or when a cardiac cause could not be excluded.

2. Re-infarction defined as recurrence of severe ischemic chest discomfort that lasted more than 20 minutes and was accompanied by recurrent ST-segment elevation of 0.1 mV in 2 contiguous electrocardiographic leads.

3. Serious arrhythmias (ventricular fibrillation and/or tachycardia).

4. Severe heart failure defined as Killip's class,[39] >II (pulmonary edema and cardiogenic shock) during hospitalization or New York Heart Association class,[40] >II after discharge.

In hospital MACE events were excluded from 30 day MACE events. Each individual MACE event was counted even if it occurred in the same patient.

Patients follow up after discharge:

All patients were seen at one month to assess LVEF and WMSI by echo and to be evaluated for MACE.

Statistical analysis of the data:

Qualitative data of the groups was analyzed using: Chi square test, Fisher Exact test, Monte Carlo test.

Normally distributed quantitative data was analyzed using: F-test (ANOVA) and Post HOC test (LSD).

While not normally distributed quantitative data was analyzed using non parametric test:

Kruskal Wallis test and Mann-Whitney test. Correlations between different parameters were done using Pearson and Spearman coefficients. P value < 0.05 was considered as significant. Statistical analysis was carried out using SPSS version 15.

Results:

There were no significant differences in baseline demographics between the 3 studied groups (table I)

Glucose Concentrations:

Glucose concentrations were similar at admission between the 3 groups; 183, 214 and 192 mg/dl at admission, in the control, IIT and GIK groups respectively. Glucose concentrations were significantly lower in IIT and GIK groups versus the Control group at 24, 48 and 72 hours after admission (table II). The reduction in glucose from the baseline was also significantly greater in these two groups after 24 hours (p=0.04), 72 hours (p=0.003), and on the mean of the 3 days (p=0.004).

The percentages of in-target RBG readings were 67.01 ± 14.61 in the Control group, 74.76 ± 17.02 in the IIT group and 68.80 ± 15.79 in the GIK group, with no statistically significant difference between the three groups (p=0.327) in terms of achievement of targets. The percentage of readings in the targeted glycemic range was significantly higher in the patients monitored using the CGM than patients monitored using 2 hours glucometer reading in all the three studied groups (56.85±12.04 [2 hrs. glucometer], 82.92±5.77 [CGM], p <0.001).

Incidence of Hypoglycemia

The incidence of hypoglycemia, defined as a blood glucose concentration below 60 mg/dl,-and severe hypoglycemia, defined as RBG below 40 mg/dl was not significantly different between the three groups.

However, there was a highly significant lower incidence of hypoglycemia in patients monitored using CGM versus those monitored every 2 hrs with a glucometer (2 in the former versus 10 in the latter, p = 0.007). The incidence of severe hypoglycemia was also significantly lower in the CGM monitored group (0 in the former and 4 in the latter group, p = 0.046). Table III)

Insulin dose:

The mean insulin dose over the three days of the study, was significantly different in the IIT and the GIK group versus the Control group (1.66 ± 0.82 units/hr [Control], 5.70 ± 3.30units/hr [IIT], 6.15 ± 3.06 units/hr [GIK], p<0.001). There was no significant difference between the insulin dose given in the IIT and the GIK group (p=0.633).

Success of thrombolytic therapy

There was no significant difference in relation to the success of thrombolytic in the three groups (p=0.928); 9 patients (60%) had successful reperfusion in the Control group, 14 patients (70%) in the IIT group and 10 patients (66.7%) in GIK group. (p=0.928). A recovery of ST elevation more than 50% on ECG performed within 90 minutes of reperfusion therapy in combination with relief of pain and the absence of electrocardiographic Q waves were considered signs of reperfusion success.

Echocardiographic Indices:

Wall Motion Score Index (WMSI) was 1.79, 2.01and 1.92 at admission, in the control, the IIT and in the GIK group respectively, with no significant difference between the 3 groups. (p=0.416). The percentage decrease in WMSI from baseline after 4 weeks was significantly greater in IIT and GIK groups than that in controls. (p=0.01) (Table IV).

Left Ventricular Ejection Fraction (LVEF%) was 38.07 ± 7.04, 36.80 ± 6.15, 36.67 ± 5.15 at admission in the control, the IIT and in the GIK group respectively, with no significant difference between the 3 groups. (p=0.76). LVEF was significantly greater after 4 weeks (p=0.003) in the IIT and GIK (p=0.037) group than in controls. The percentage increase in LVEF from baseline in both IIT and GIK groups after 72 hours (p=0.049) [data not shown] and 4 weeks (p=0.002) was significantly greater than that in control group (Table IV).

Plasma hsCRP Concentrations:

Plasma hsCRP concentrations was 1.98±0.69 mg/L, 1.98±0.68 mg/L and 2.08±0.74 mg/L in the control, IIT and GIK groups respectively, at admission (p=0.898). CRP concentrations were significantly lower at 48 hrs in IIT and GIK groups than those in the control group (p<0.001). The increase in hsCRP from the baseline was also significantly

lower in IIT and the GIK groups versus the control group (p<0.001). (Table V).

Plasma creatinine kinase (CK) Concentrations:

On admission serum CK levels; 518.20±485.08 mg/L in the control group, 349.50±386.88 mg/L in the IIT group and 316.57±201.10 mg/L in the GIK group. (p =0.303). There was a statistically significant increase in the peak CK value in the control group versus the IIT and the GIK group (p = 0.03), with no significance between the IIT vs the GIK group (p=0.730). (Table V)

Major adverse cardiac events (MACEs)

The incidence of in-hospital MACE was significantly different between the three studied groups (p=0.05), with a higher incidence in the control versus the IIT group, (p=0.022). (Table VI).

The incidence of in-hospital serious arrhythmias (ventricular fibrillation and/or tachycardia) and severe heart failure were significantly higher in the control versus the IIT group (p = 0.027). There was no difference between the incidences of in-hospital re-infarction and cardiac death between the three studied groups (Table VI).

There was also a significant difference between the three studied groups in the incidence of 30 day MACEs (p=0.015), with a significantly higher incidence in the control versus the IIT group and the GIK group (p=0.022, 0.035, respectively).

At 30 days, the incidence of severe heart failure was significantly higher in the control versus the IIT and the GIK group (p = 0.022, 0.035, respectively). There were no significant differences in the incidences of 30 day serious arrhythmias, re-infarction and cardiac death between the three groups. (Table VI).

Comparing IIT and GIK

When comparing the IIT and the GIK group, we found no benefit from the infusion of 25% Glucose in the GIK group. The incidence of hypoglycemia (p=0.7) and severe hypoglycemia (p=1.0) showed no statistically significant difference between the two groups. At the clinical level, there were no significant differences at 4 weeks on the incidence of MACE (p=1.0), improvement of the myocardial contractility (WMSI p=0.448 & EF% p=0.421), the attenuation of the increase of hsCRP (p=0.944).

Table I: Studied Groups Characteristics

	Control (n=15)		IIT (n=20)		GIK (n=15)		p
	No.	%	No.	%	No.	%	
Gender							
Male	10	66.7	12	60.0	9	60.0	0.906
Female	5	33.3	8	40.0	6	40.0	
Weight	83.93 ± 13.01		82.80 ± 13.81		84.40 ± 17.74		0.888
Smokers	7	46.7	10	50.0	8	53.3	0.936
Diabetics	8	53.3	10	50.0	9	60.0	0.840
Hypertensive	5	33.3	12	60.0	7	46.7	0.293
Anterior MI	5	33.3	6	30.0	5	33.3	0.970
Extensive Ant. MI	4	26.7	7	35.0	5	33.3	0.865
Lateral MI	5	33.3	7	35.0	6	40.0	0.924
Inferior MI	6	40.0	6	30.0	4	26.7	0.714
Pain to needle time (mins) Mean ± SD	224.33 ± 1.06.43		235.35 ± 95.38		227.00 ± 99.46		0.951
Door to needle time (mins) Mean ± SD	30.33 ± 15.29		31.35 ± 14.48		28.67 ± 12.60		0.833

IIT: Intensive Insulin Therapy group
GIK: Glucose Insulin Potassium Group
MI: Myocardial Infarction

Table II: Comparison between the different studied groups according to random blood glucose (RBG) (mg/dl) on admission, mean 1st, 2nd, 3rd and three days, mean Insulin dose over first 24 hours (U/hr), and over 72 hours (U/hr), IV Glucose 1st 24h (g/hr), incidence of hypoglycemia and severe hypoglycemia.

	Control (n=15)	IIT (n=20)	GIK (N=15)	p	p_{1a}	p_{1b}	p_2
RBG (mg/dl)							
On admission	182.73 ± 81.19	214.30± 108.12	192.40 ± 84.58	0.782	0.494	0.740	0.714
Mean 1st day	146.60 ± 12.89	118.02 ± 19.79	118.79 ± 17.41	<0.001	<0.001	<0.001	0.9
Mean 2nd day	145.57 ± 12.47	106.90 ± 13.57	110.57 ± 15.45	<0.001	<0.001	<0.001	0.45
Mean 3rd day	143.07 ± 9.61	108.93 ± 13.40	111.56 ± 19.12	<0.001	<0.001	<0.001	0.6
Mean 3 days	145.08 ± 9.14	111.29 ± 13.45	113.64 ± 15.11	<0.001	<0.001	<0.001	0.601
Insulin 1st 24h (U/hr)	1.59 ± 0.79	5.08±3.09	7.87±2.85	<0.001	<0.001	<0.001	0.019
Insulin 72 h (U/hr)	1.66 ± 0.82	5.70 ± 3.30	6.15 ± 3.06	<0.001	<0.001	<0.001	0.633
IV Glucose 1st 24h (g/hr)	0.42 ± 0.39	1.70± 0.85	21.1±5.5	<0.001	<0.001	<0.001	<0.001
Hypoglycemia	2 (13.3%)	5 (25.0%)	5 (33.3%)	0.506	0.672	0.390	0.712
S. hypoglycemia	0 (0.0%)	2 (10.0%)	2 (13.3%)	0.543	0.496	0.483	1.000

IIT: Intensive Insulin Therapy group

GIK: Glucose Insulin Potassium Group

RBG: Random Blood Glucose

S. hypoglycemia: severe hypoglycemia

p: p value comparing between the three studied groups

p1a: p value comparing between Control group and the IIT group.

p1b: p value comparing between Control group and the GIK group.

p2: p value comparing between IIT group and the GIK group.

Table III: Comparison between the two methods of monitoring random blood glucose (RBG) according to the percent of in target RBG readings, incidence of hypoglycemia and severe hypoglycemia

	2 hrs	CGM	p
% of In-Target RBG Readings	56.85±12.04	82.92±5.77	<0.001
Hypoglycemia	10 (41.7%)	2 (7.7%)	0.007
S. hypoglycemia	4 (16.7%)	0 (0%)	0.046

2 hrs: Method of monitoring random blood glucose using capillary blood glucometer device every 2 hours interval.

CGM: Method of monitoring random blood glucose using continuous glucose monitoring device.

RBG: Random Blood Glucose

S. hypoglycemia: severe hypoglycemia

Table IV: Comparison between the different studied groups according to ejection fraction (EF %) & wall motion score index (WMSI)

	Control	IIT	GIK	p	p_{1a}	p_{1b}	p_2
EF%							
On admission	38.07 ± 7.04	36.80 ± 6.15	36.67 ± 5.15	0.76	0.484	0.566	0.932
At 72 hours	44.33 ± 8.42	49.85 ± 9.02	47.50 ± 7.77	0.174	0.064	0.321	0.432
After 1 month	43.87 ± 11.27	55.65 ± 11.01	52.57 ± 10.23	0.009	0.003	0.037	0.421
% of change after 1 month	15.92 ± 24.00	53.00 ± 31.35	44.34 ± 30.39	0.002	0.001	0.009	0.285
WMSI							
On admission	1.79 ± 0.46	2.01 ± 0.51	1.92 ± 0.47	0.416	0.188	0.472	0.577
At 72 hours	1.56 ± 0.39	1.46 ± 0.40	1.49 ± 0.41	0.785	0.491	0.672	0.822
After1 month	1.55 ± 0.38	1.33 ± 0.44	1.34 ± 0.38	0.117	0.059	0.131	0.448
% of change after 1 month	-12.32 ± 10.83	-31.15 ± 20.27	-28.81 ± 17.78	0.013	0.010	0.010	0.753

IIT: Intensive Insulin Therapy group
GIK: Glucose Insulin Potassium Group
p: p value comparing between the three studied groups
p1a: p value comparing between Control group and the IIT group.
p1b: p value comparing between Control group and the GIK group.
p2: p value comparing between IIT group and the GIK group.

Table V: Comparison between the different studied groups according to High sensitivity C-reactive protein measurement (hsCRP) (mg/L), Creatine Kinase (CK) levels (mg/L).

	Control (n=15)	IIT (n=20)	GIK (n=15)	p	p_{1a}	p_{1b}	p_2
hsCRP(mg/L)							
On admissions	1.98 ± 0.69	1.98 ± 0.68	2.08 ± 0.74	0.898	0.998	0.696	0.678
After 48 hours	11.31 ± 2.95	4.76 ± 0.82	4.79 ± 0.76	<0.001	<0.001	<0.001	0.944
% of change	-10.15 ± 27.55	-40.90 ± 20.98	-34.01 ± 23.48	<0.001	<0.001	<0.001	0.506
CK (mg/L)							
On admissions (mean)	518.20 ± 485.08	349.50±386.88	316.57 ± 201.10	0.303	0.201	0.161	0.805
On admissions (median)	376	251	301	0.461	0.25	0.351	0.714
Peak value (mean)	3136.2 ± 902.29	2393.70±868.38	2495.79±732.24	0.034	0.013	0.047	0.730
Peak value (median)	3154	2595	2198.5	0.05	0.029	0.04	0.806

IIT: Intensive Insulin Therapy group
GIK: Glucose Insulin Potassium Group
hs CRP: High sensitivity C-reactive protein
CK: Creatine kinase
p: p value comparing between the three studied groups
p1a: p value comparing between Control group and the IIT group.
p1b: p value comparing between Control group and the GIK group.
p2: p value comparing between IIT group and the GIK group.

Table VI: Comparison between the different studied groups according to incidence of in-hospital major adverse cardiac events (MACEs) & discharge to 30 days follow up MACEs

		Control (n=15)		IIT (n=20)		GIK (n=15)		p	p_{1a}	p_{1b}	p_2
		N	%	N	%	N	%				
IN HOSPITAL MACEs	**Serious arrhythmia**	6	40.0	1	5.0	2	13.3	0.028	0.027	0.215	0.565
	Heart Failure	6	40.0	1	5.0	2	13.3	0.028	0.027	0.215	0.565
	Re-infarction	0	0.0	0	0.0	0	0.0	-	-	-	-
	Death	0	0.0	0	0.0	1	6.7	0.598	-	1.000	0.429
	All MACEs (subjects)	7	46.7	2	10.0	4	26.7	0.05	0.022	0.45	0.367
Discharge to 30 days follow up MACEs	**Serious arrhythmia**	0	0.0	0	0.0	0	0.0	-	-	-	-
	Heart Failure	7	56.7	2	10.0	1	7.1	0.015	0.022	0.035	1.000
	Re-infarction	1	6.7	0	0.0	0	0.0	0.591	0.429	1.000	-
	Death	1	6.7	0	0.0	0	0.0	0.591	0.429	1.000	-
	All MACEs (subjects)	7	46.7	2	10.0	1	7.1	0.015	0.022	0.035	1.000

IIT: Intensive Insulin Therapy group
GIK: Glucose Insulin Potassium Group
p: p value comparing between the three studied groups
p1a: p value comparing between Control group and the IIT group.
p1b: p value comparing between Control group and the GIK group.
p2: p value comparing between IIT group and the GIK group.

Appendix 1: Sliding Scale Protocol with Regular Insulin SC:

RBS (mg/dl)	Low Dose Scale	Moderate Dose Scale	High Dose Scale	Next Glucose Check
Below 60	**administer 100cc G10% IV bolus and re-measure after 30 mins.**			30 mins
60-130	0 units	0 units	0 units	6 hours
131-180	2 units	4 units	8 units	6 hours
181-240	4 units	8 units	12 units	6 hours
241-300	6 units	10 units	16 units	6 hours
301-350	8 units	12 units	20 units	6 hours
351-400	10 units	16 units	24 units	6 hours
Above 400	12 units	20 units	28 units	6 hours

Discussion:

Our data show clearly that in patients with STEMI, tight glycemic control (BG < 120mg/dl) with continuous insulin infusion either administered as insulin alone or in combination with high dose GIK leads to a greater improvement in the absolute ejection fraction at 30 days, than that in subjects with BG > 140mg/dl. In addition, the wall motion index, reflective of myocardial systolic function, was also improved more in the

intensively insulin treated patients than that in controls. These observations are consistent with a significant reduction in myocardial injury and probably a reduction in the size of the infarct. Consistent with this, there was a smaller magnitude of increase in CK concentrations in the IIT and GIK groups when compared to the controls. In addition, the patients in the IIT and GIK groups had smaller increases in plasma hsCRP concentrations which have previously been shown to be related to myocardial injury [41-43]. At the clinical level, intensively treated patients had fewer MACE including dysrythmia and cardiac failure. Thus, intensive insulin treatment exerted anti-inflammatory and cardio-protective effects while improving clinical outcomes. These effects are consistent with the observations recently published by Chaudhuri et al. [44].

Our study also demonstrates for the first time that with higher rates of glucose and insulin infusion as in the GIK group, if blood glucose concentrations are maintained between 80-120 mg/dl, there is a reduction in the magnitude of increase in CK and hsCRP concentrations along with an improvement in ejection fraction when compared with controls. These results were similar to those observed in the IIT group and are also consistent with those reported by Chaudhuri et al. [44]

In view of the absence of overt benefit from fixed high dose insulin and glucose regimes in the past, as in the CREATE-ECLA study, [24] the critical factor necessary for obtaining benefit with all such regimes appears to be the maintenance of euglycemia. In the CREATE-ECLA study, the institution of the GIK regime resulted in the induction of hyperglycemia consistent with the large amounts of glucose (25-30 g/h) infused: glucose concentrations increased from 162 mg/dl to 182 mg/dl at 6h and remained higher than that in controls throughout the period under study (24h). It is also of interest that in a re-analysis of the CREATE-ECLA study, it was clear that there was an excess of mortality and cardiac failure in the first 3 days of admission in the GIK arm while there was a reduction in mortality and cardiac failure between days 3-30. This is consistent with the detrimental effects of induction of hyperglycemia and fluid overload immediately after admission with insulin related benefits appearing in the later phase (days 3-30). In a further analysis of the data from this study, it was shown clearly that for any given concentration of glucose, the group given insulin had lower mortality when glucose concentrations were higher than 144mg/dl [45].In other previous regimes using high glucose and insulin infusion rates also, no effort was made to maintain euglycemia. In our study, we were able to maintain mean glucose concentrations at 108 mg/dl in the IIT arm and at 112 mg/dl in the GIK arm when compared to 143 mg/dl in the control arm.

Our data also show that blood glucose concentrations greater than 140 mg/dl are detrimental in patients with AMI. These observations are consistent with that of the HI-5 Study. [25] In the HI-5 study, there was a 50% reduction in the incidence of cardiac failure and re-infarction; however, there was no reduction in the rate of mortality in the insulin infused group. This was probably related to the minimal difference in blood glucose concentrations between the two groups: 8.3 ± 2.2 mmol/L (149.4 ± 39.6 mg/dl), in the insulin infusion group, and 9.0 ± 2.8 mmol/L (162 ± 50.4 mg/dl) in the control group (p=NS). When the data were analyzed on the basis of mean blood glucose concentrations achieved during the first 24 h, mortality was lower among patients with a mean blood glucose concentration of ≤ 8 mmol/L (144 mg/dl), compared with patients with a mean blood glucose concentration of >8 mmol/L (2 vs. 11% at 6 months, p = 0.02). Our observations are also consistent with that of Marfella et al [46] who have shown that in patients with first AMI, undergoing coronary bypass surgery, tight glycemic control (blood glucose 80-140 mg/dl) with insulin for three days before surgery, reduces oxidative stress and inflammation, reduces apoptosis and remodeling in the peri-infarct zones . These effects are associated with a reduction in myocardial infarct size and an improved left ventricular ejection fraction.

These observations may explain why there was no difference in mortality between the three groups in the DIGAMI-2 study(26). In that study, all groups had mean glucose concentrations greater than 140 mg/dl and the decrease in glucose concentrations achieved in the insulin infused arms was not significant. In contrast, in the DIGAMI 1 study, (27) which

showed a benefit of insulin infusion, there was a significant reduction in glucose concentrations in the insulin treated group compared to controls.

The present study also demonstrates for the first time that the continuous glucose monitoring system can be used effectively in studies investigating the effect of tight glycemic control on clinical outcomes in AMI. The percentage of in-target glycemic readings was significantly higher in the patients monitored using the CGM than patients monitored using glucometer readings (56.85±12.04 [2hours glucometer], 82.92±5.77 [CGM], p <0.001). Patients monitored using the CGM system reached a significantly lower glycemic level in the first 24 hours (134.44 ± 22.31 [2hours Glucometer], 120.39 ± 18.62 [CGM], p=0.02) This lower glycemic level was maintained when computing the mean glycemic level for 72 hours (130.41 ± 19.80 [Glucometer], 115.13 ± 17.16 [CGM], p=0.006) (data not shown) .Indeed, in our study, we achieved the best glycemic levels of any study investigating the effects of insulin infusion in patients with AMI.

Increased incidence of hypoglycemia is a major concern when implementing tight glycemic control. In our study there was a highly significant reduction in the incidence of hypoglycemia in the CGM group (10 [2 hrs GlucoCheck], 2 [CGM], p = 0.007). Furthermore, none of the patients monitored using the CGM system encountered any episode of severe hypoglycemia The reduction in hypoglycemia with CGMS is an important observation since recent studies, like the NICE-SUGAR study(47), tight glycemic control in the ICU was shown not to be beneficial. In this study there was a significantly higher incidence of severe hypoglycemia in the intensive glucose control (6.8%) versus the conventional control group (0.5%), As hypoglycemia is associated with adverse clinical outcomes and is also a pro-inflammatory and prothrombotic stimulus (48-50) higher incidence of hypoglycemia in the intensive group may have neutralized the benefits of insulin infusion and tight glycemic control in these studies. Thus, it is important that in any trial investigating the benefits of tight glycemic control, hypoglycemia is avoided. Continuous glucose monitoring allows easier treatment of hyperglycemia and the prevention and the prompt treatment of hypoglycemia. It should, therefore, be possible in the future to conduct such studies effectively, safely and in a simpler fashion by using CGMS to monitor glucose.

Conclusion: intensive insulin infusion treatment along with continuous glucose monitoring allowed us to maintain blood glucose concentrations at around a mean of 108 mg/dl in the IIT group and 112 mg/dl in the GIK group compared to 143 mg/dl in the control group over a period of 3 days without significant increase in the hazard of hypoglycemia. This resulted in significantly reduced incidence of MACE (dysrhythmia and cardiac failure), less myocardial dyskinesia and an improved ejection fraction, and lower CK and CRP concentrations in patients with STEMI. Future large multicenter trials need to be conducted with such insulin regimens targeting tight glycemic control using continuous glucose monitoring to confirm our findings in patients with STEMI and to establish the use of intravenous insulin infusions in the treatment of patients with this condition.

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Original Article

Osteoprotegerin, High Sensitive C-Reactive Protien, Insulin Resistance and Their Relation to Carotid Intima Media Thickness in Type2 Diabetic Subjects.

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Abstract:

Background: The role of Osteoprotegerin (OPG) as a marker of atherosclerosis in (T2DM) is not well established. Moreover, the relationship between OPG and insulin resistance in T2DM remains to be elucidated. **Aim:**To determine the role of serum OPG in the prediction of atherosclerosis and insulin resistance in T2DM males. **Subjects and Methods:** Cross-sectional study with 80 males, 40 with T2DM and 40 subjects without diabetes and (ACVD) was excluded in both groups. We measured: serum OPG by immunoassay, FBG, HbA1C, HOMA-IR, lipid profile, Albumin/creatinine ratio, hs-CRP, carotid intima- media thickness (IMT) and ankle brachial index (ABI). The presence of ACVD was excluded by a negative stress test and/or coronary angiography. **Results:** The mean serum OPG level was statistically significantly higher in the diabetic group compared to the control group (p =0.029*),the mean serum OPG level was 506.45±488.48 ng/ml in the diabetic group and 266±272.73 ng/ml in the control group.OPG was statistically significantly positively correlated to age (p=0.047) also with HbA1c (p=0.009) and DM duration (p=0.041). However, OPG was not related to smoking, IMT, ABI or level of albuminuria. **Conclusions:** Our study showed that T2DM patients had higher OPG serum levels with respect to the non-diabetic population, butthe relationship of OPG and atherosclerosis in T2DM patients needs further investigations.

Keywords: Osteoprotegerin, Hs CRP, IR, T2 DM

Introduction:

Diabetes mellitus is a complex metabolic condition primarily defined by the level of chronic hyperglycemia giving rise to risk of vascular complications that may be microvascular or macro vascular.

Diabetes is a chronic illness and needs continuous medical care with multifactorial risk reduction strategies beyond glycemic control.(1) Atherosclerotic cardiovascular disease (ACVD) is the major cause of morbidity and mortality in T2DM patients.T2DM patients have (2-4) times increased risk of cardiovascular events with respect to subjects without diabetes.(2) (OPG) is a promising marker for atherosclerosis in patients with T2DM and insulin resistance. (OPG) is a glycoprotein that acts as a decoy receptor for receptor activator of nuclear factor B ligand (RANKL) and TNF-related apoptosis-inducing ligand. The OPG/RANKL/ receptor activator of nuclear factor B axis plays an important regulatory role in the skeletal, immune, and vascular systems.At the vascular level OPG is expressed in vivo by endothelial cells (ECs), vascular smooth muscle cells (VSMCs). RANKL is expressed in vivo by stromal cells and T- lymphocytes.(3)

Abbreviations: ABI: Ankle/brachial index, **ACVD:** Atherosclerotic cardiovascular disease**, BMI:** Body mass index, **CAD:** coronary artery disease, **CVS:** cerebrovascular disease, **DM:** Diabetes mellitus, **ECs:** Endothelial cells, **HbA1c:** Haemoglobin A1c, **HDL-C:** High Density Lipoproteins, **HOMA-IR:** Homeostasis Model Assessment insulin resistance, **hs-CRP:** high sensitive-C-reactive protein**, I-Kb:** Inhibitory kappa B, **IMT:** intima media thickness, **LDL-C:** Low Density Lipoproteins cholesterol, **NSTEMI:** non-ST segment elevation myocardial infarction, **PAD:** peripheral arterial disease, **RANK:** Receptor activator of nuclear factor k-B, **RANKL:** Receptor activator of nuclear factor k-B ligand, **STEMI:** ST segment elevation myocardial infarction, **VSMCs:** Vascular smooth muscle cells.

RANK is expressed on the surface monocytes, macrophages and dendritic cells.[4,5] Interaction of RANKL with RANK activates nuclear factor I-kB (a transcription factor), degradation of I-kB protein by I-kB kinase; frees the nuclear factor-kB complex, which then translocate to the nucleus initiating transcription of specific genes required for differentiation of macrophages.[6]

There is association between OPG levels and traditional cardiovascular risk factors.[6, 7] The major consistent direct association seems to be with increasing age and duration of diabetes. [7]The association of serum OPG levels with markers of endothelial dysfunction has been reported in patients with T1DM and T2DM. [8, 9]

In a general population serum OPG is associated with future risk of myocardial infarction, ischemic stroke and total mortality independent of other cardiovascular risk factors.[10] It has also been shown that OPG serum levels are related to increased cardiovascular mortality in accelerated atherosclerosis related condition conditions as advanced age[11] and CAD.[4]

Avignon et al[12] demonstrated the significant association of OPG levels and silent ACVD, diagnosed by myocardial perfusion imaging in patients with T2DM. The increase in OPG in T2DM has been linked with higher risk of coronary artery calcification [13] and silent CAD. [12] Serum OPG concentrations were predictive of increased risk of cardiovascular events in T2DM during an 18-month follow-up.[7] Reinhard et al[14] demonstrated that elevated serum OPG is a strong predictor of all-cause mortality in T2DM patients. This effect is independent of conventional cardiovascular risk factor.

Patients and Methods:

This cross-sectional study was conducted on 80 male subjects divided into two groups. Group I: 40 T2DMpatients without CAD.Group II: 40 Healthy control subjects matched for age, sex and socioeconomic status.

CAD was excluded in group I by a negative stress test and/or coronary angiography. Subjects of group I were recruited from those undergoing coronary angiography and/or stress test for a justified indication at the cardiology department, Alexandria University, and proved to be free of CAD. Patient categorization was based on history, clinical, laboratory, stress test and or coronary angiography.Patients with the following associated conditions were excluded from the study: history of acute coronary syndrome, unstable angina, NSTEMI and STEMI, history of infection with in the last 2 months, endocrinal, metabolic diseases or immunological diseases other than DM and T1DM.After giving their signed informed consent; all the participants were subjected to the following:full history taking in which personal history included name, age and occupation, history of cigarette smoking: according to smoking status, participants were classified into:

- Non-smoker: those with a lifetime exposure to no cigarettes.
- History of smoking: Including current and ex-smokers.

History of different cardio-metabolic risk factors: DM, hypertension, dyslipidaemia, drughistory and family history: participants were asked about family history of CAD, CVS, PAD, hypertension, dyslipidaemia and DM.

Complete physical examination with special stress on: body weight and height, BMI and waist circumference, vitalsigns: (heart rate and arterial blood pressure), andassessment of ABI.

Laboratory Assessment Including:

FBG,CBC, Fasting serum insulin level, HbA1C, Total serum cholesterol, HDL-C,LDL-C, Serum triglycerides, hs-CRP and serumOPG level using ELISA assays kit. Venous blood samples (10 ml from each patient) were collected from antecubital vein into vacuum tubes with appropriate additives. Sampling was done in the morning (8.00 - 10.00 am) of the same day of the Cardiac Catheterization and coronary angiography after an overnight fast of 12 hours. All subjects were asked to refrain from smoking and strenuous exercise during the fasting period. The collected venous samples were divided into 2 parts, one part in plain vacutainer tube left to clot at 37◦C: sera were separated by centrifugation and divided into 2 parts, one used for immediate assay of fasting glucose, insulin level, lipid profile and

hs-CRP. The other was kept at -70◦C for assay of OPG. The other part of blood sample was collected on EDTA tube for complete blood count and HbA1c.

Other investigations: Resting ECG, coronary angiography and Doppler Carotid Artery to determine the carotid IMT. Ultrasonographic scanning of the carotid arteries was performed using Philips ClearVue 350 at a transducer frequency of 7-12 MHz at Radiology Department, Alexandria Main University Hospital. Extracranial carotid arteries in the neck were scanned bilaterally in the longitudinal and in the transverse plane. This provided images of the common carotid artery, the carotid bulb, and parts of the internal and external carotid arteries. Scanning was performed by experienced ultrasonographers.

Statistical Analysis of the Data:

Data were fed to the computer and analysed using IBM SPSS software package version 20.0. Qualitative data were described using number and per cent. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level. The used tests were **Chi-square test:** for categorical variables, to compare between different groups, **Fisher's Exact or Monte Carlo correction:** correction for chi-square when more than 20% of the cells have expected count less than 5, **Student t-test:** for normally quantitative variables, to compare between two studied groups and **Mann Whitney test:** For abnormally quantitative variables, to compare between two studied groups.

Results:

Anthropometric measures: Table (I)

The mean waist circumference wasstatistically significant much higher in the diabetic group than the control group, (p= 0.015). Also, there was a statistically significant difference regarding the waist to hip ratio being higher in the diabetic group than in the control group, (p=0.005).

Arterial blood pressure: Table (II):

The mean value of the systolic blood pressure was statistically significant higher in the diabetic group than the control group, (p=0.009). The mean value of the diastolic blood pressure was statistically significant higher in the diabetic group than the control group, (p=0.039). The mean value of the ABI was statistically significant lower in the diabetic group than the control group, (p=0.001).

Lipid profile: Table (III)

The mean value of HDL-C was statistically significant lower in the diabetic group than in the control group, (p=0.049).

Fasting plasma glucose, HbA1c and HOMA2-IR: Table (IV):

The mean fasting plasma glucose was higher in the diabetic group than in the control group, (P=0.000). The mean HbA1c percentage was higher in the diabetic group than the control group, (P<0.001).The mean HOMA2-IR value There were no statistically significant differences between both groups regarding the mean HOMA2-IR value, (p=0.946).

Hs-CRP, OPG: (Table V, figures1):

The mean serum hs-CRP level was higher in the diabetic group than in the control group, (p<0.001). The mean serum OPG level was higher in the diabetic group than in the control group, (p=0.029).

Carotid (IMT):

The mean IMT was higher the diabetic group than in the control group, (p<0.001).

Serum OPG was statistically significantly positively correlated to age (p=0.047), also with HbA1c (p=0.009) as shown at **Figure (2)**, and DM duration (p=0.041) as shown at **Figure (3)**.

There were no statistically significant correlations between serum OPG and carotid (IMT). (p=0.581). There were no statistically significant correlations between serum OPG and hs-CRP. (p=0.114).There was no statistically significant difference between the mean serum OPG levels in the diabetic patients with and without albuminuria, (p=0.613).There was no statistically significant difference between the mean serum OPG levels in those with or without history of smoking in the studied groups, (p=0.137).

Table (I): Comparison between the two groups as regards anthropometric measurements

	Group I (n = 40)	Group II (n = 40)	T	P
Waist circumference (cm)				
Range	92.0 – 108.0	89.0 – 115.0	2.487*	0.015*
Mean ± SD	100.60 ± 4.61	97.50 ± 6.39		
Waist/hip ratio				
Range	0.93 – 1.04	0.85 – 0.99	2.912*	0.005*
Mean ± SD	0.97 ± 0.02	0.95 ± 0.03		

t: Student t-test
P≤ 0.05 = statistically significant
P> 0.05= statistically non-significant

Table (II): Comparison between the two groups as regards SBP, DBP & ABI

	Group I (n = 40)	Group II (n = 40)	t	P
Systolic blood pressure (mmHg)				
Mean ± SD	130.63 ± 12.31	123.12 ± 12.54	2.699*	0.009*
Diastolic blood pressure (mmHg)				
Mean ± SD	83.0 ± 10.61	78.50 ± 8.41	2.102*	0.039*
Ankle brachial index				
Mean ± SD	0.99±0.08	1.05±0.10	3.294*	0.001*

t: Student t-test
P≤ 0.05 = statistically significant
P> 0.05= statistically non-significant

Table (III): Comparison between the two groups as regards lipid profile

	Group I (n = 40)	Group II (n = 40)	t	P
HDL-C (mg/dl)				
Mean ± SD	36.53 ± 13.45	43.29 ± 16.44	1.969*	0.049*

t: Student t-test
P≤ 0.05 = statistically significant
P> 0.05= statistically non-significant

Table (IV): Comparison between the two groups as regards FPG, HbA1c, Serum insulin& HOMA2-IR

	Group I (n = 40)	Group II (n = 40)	t	P
Fasting blood sugar (mg/dl)				
Mean ± SD	140.65 ±76.08	92.45 ± 18.92	2.779*	0.000*
HbA1c (%)				
Mean ± SD	8.59±1.96	5.72±0.53	6.778*	<0.001*
HOMA2-IR				
Mean ± SD	1.67 ± 1.68	1.54 ± 1.21	0.067	0.946

t: Student t-test
P≤ 0.05 = statistically significant
P> 0.05= statistically non-significant

Table (V): Comparison between the two groups as regards hs- CRP and OPG

	Group I (n = 40)	**Group II (n = 40)**	**T**	**P**
Hs-CRP(mg/L) Mean ± SD	12.32 ± 11.24	5.29 ± 6.09	3.822*	<0.001*
OPG (ng/ml) Mean ± SD	506.45±488.48	266±272.73	2.180	0.029*

t: Student t-test

P≤ 0.05 = statistically significant

P> 0.05= statistically non-significant

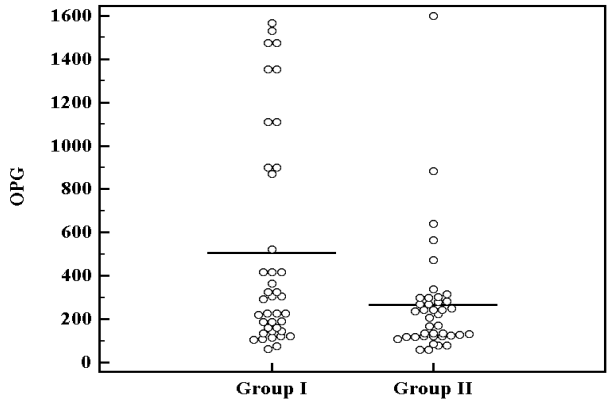

Figure (1): Comparison between the two groups as regards OPG

Table (VI): Comparison between the two groups regarding carotid intima media thickness

	Group I (n = 40)	**Group II (n = 40)**	**t**	**P**
IMT(mm) Mean ± SD	0.96±0.20	0.69±0.15	6.740	<0.001*

t: Student t-test

P≤ 0.05 = statistically significant

P> 0.05= statistically non-significant

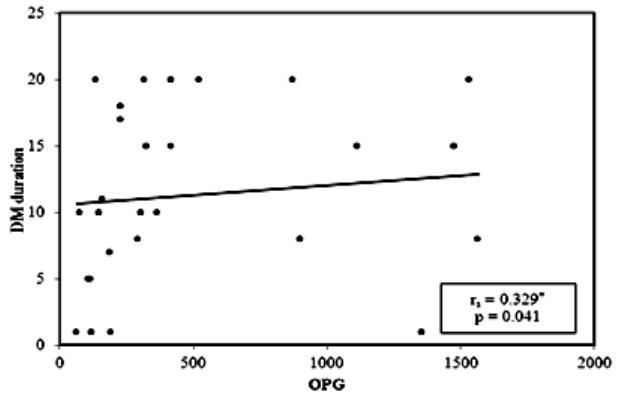

Figure (2): Correlation between OPG and HBA1c (%) in total sample.

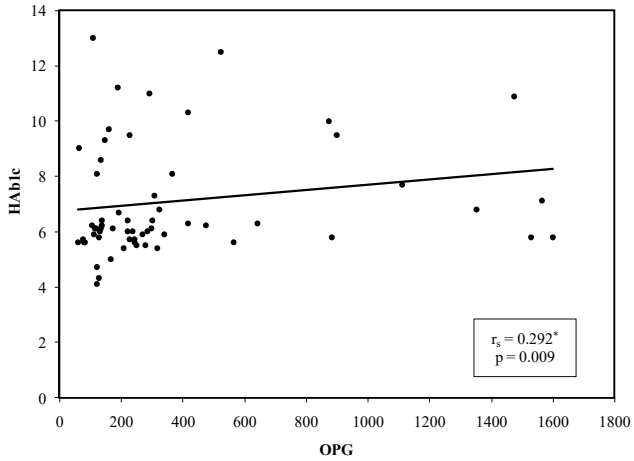

Figure (3): Correlation between OPG and diabetes duration (years) in total sample.

Discussion:

In the present study, the mean value of the waist circumference was statistically significantly higher in the diabetic group compared to the control group (p=0.015). Also, there was a statistically significant difference regarding the waist to hip ratio being higher in the diabetic group than in the control group, (p=0.005).Our results agree with published data by Björntorp et al that showed Visceral obesity is a strong predictor of T2DM and is associated with insulin resistance. Data suggest that visceral accumulation of fat contributes to increased circulating levels of FFA that cause insulin resistance and development of T2DM. [15]

The current study showed that. Both systolic and diastolic BP are much higher in T2DM than non-diabetic patients. Our results agree with data described by Buse et al [16] that showed the positive correlation between T2DM and hypertension, which was explained by the fact that hypertension is generally attributed to hyperinsulinemia resulting in increase in renal sodium retention and/or sympathetic nervous system activity [17]. Also, Insulin has an important role in the modulation of cellular calcium metabolism. Decreased insulin action on vascular smooth muscle cells may contribute both to hypertension and to accelerated atherosclerosis. [18]

In the present study, the mean value of the ABI was statistically significantly lower in the diabetic group compared to the control group (p=0.001). ABI is a non-invasive screening tool essential for diagnosing PAD; it measures clinical bedside atherosclerotic burden and risk of future CVD events. ABI is lower in the diabetic group than the control group which confirms that development of PAD is more common in patients with diabetes than non-diabetics, which agrees with Lüscher et al [19] who showed that PAD is a strong independent predictor of cardiovascular morbidity and all-cause mortality, and is a common manifestation of systemic atherosclerosis.

The results of the present study showed that there were no significant differences regarding total cholesterol, LDL-C and TG between both groups, however, the mean value of HDL-C was statistically significantly lower in the diabetic group compared to the control group, (p =0.049), which is in agreement with previous data described by Singh et al [20] and Barter P[21] who showed that HDL-C has several protective properties that are independent of their involvement in cholesterol metabolism, for example, they reduce oxidation, vascular inflammation and thrombosis, improve endothelial function, promote endothelial repair, enhance insulin sensitivity and promote insulin secretion by pancreatic beta islet cells.

In the present study, the mean carotid IMT was statistically significantly higher in the diabetic group than in the control group, (p=<0.001) which is in agreement with us, Ishiyama et al[22] who demonstrated that diabetic patients had much higher carotid IMT.

In the current study, the mean serum hs-CRP level was statistically significantly higher in the diabetic group compared to the control group (p<0.001*). This is in accordance with previous data prescribed by

Festa et al [23] who reported that increased hs-CRP is associated with insulin resistance. Hs-CRP has been proved to be a marker of chronic subclinical inflammation, to be a predictor of cardiovascular events, and to be independently related to Insulin resistance.

In the present study, the mean serum OPG level was statistically significantly higher in the diabetic group compared to the control group (p =0.029*) which agrees with the data from the previous study conducted by Knudsen et al [24] who showed that plasma OPG was significantly higher in diabetic patients.

In the present study, serum OPG level was statistically significantly positively correlated to age (p=0.047), that agrees with previous reports conducted by Gannagé-Yared et al [6] and Anand et al [7] that demonstrated serum OPG levels were significantly correlated with increasing age.

In the present study, serum OPG level was statistically significantly positively correlated to HbA1c (p=0.009) and DM duration (p=0.041) which agrees with previous reports described by Shin et al [9] demonstrated that serum OPG levels increase with age, diabetes and is positively correlated glycaemic status, and insulin resistance.

In the present study, serum OPG level was not correlated with carotid IMT. Our results agree with the study Vik et al [25] who demonstrated that increased serum OPG does not promote early atherosclerosis in younger subjects.

On the other hand, Akinci et al [26] demonstrated that elevated serum OPG levels have significant correlation with carotid IMT, which suggested that OPG might play a role in the pathogenesis of endothelial dysfunction.

In the present study, serum OPG level was not correlated to ABI, which is not in agreement with Ziegler et al [27] who reported a positive correlation between serum levels of OPG and severity of PAD. The explanation of those results is that Ziegler et al studied serum OPG level in advanced symptomatic PAD in lower extremities and their results demonstrated that serum OPG concentrations were significantly higher in subjects with ischemic ulcerations than in those without and were positively correlated with higher severity grade of disease and age .On the other hand, Pennisi et al [28] reported that there was no statistically significant difference in OPG levels between patients with documented PAD and healthy controls.

In the present study, serum OPG level was not correlated with hs-CRP which agrees with Anand et al [7] who showed that there was no significant correlation between hs-CRP and serum OPG level and it was explained by serum OPG level didn't predict cardiovascular mortality in short term.

In the present study, level of serum OPG not correlated with albuminuria.However, Wang et al [29] demonstrated that serum OPG level and the OPG expression in renal tubule cells of biopsy tissue were increased in nephropathy of T2DM.

The explanation of those results is that no patients in the current study had ACVD and the process of atherosclerosis is too early, so further investigations are needed to correlate the serum level of OPG to other atherosclerosis markers in patients with established ACVD.

On the other hand, our study demonstrated positive correlation between T2DM, duration of DM and elevated serum level of OPG.

Conclusion:

Our study showed that T2DM patients had higher OPG serum levels with respect to the non-diabetic population, but the relationship of serum OPG and atherosclerosis in T2DM patients needs further investigations.

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Original Article

Assessment of High Sensitivity Cardiac Troponin-T in Chronic Kidney Disease Patients.

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Abstract:

Background: High sensitivity cardiac troponin T is associated with cardiovascular events and mortality in patients with chronic kidney disease (CKD). Serum levels of high sensitivity cardiac troponin T (hs-cTnT) reflect subclinical myocardial injury in CKD patients. We sought to determine the role of hs-cTnT in CKD patients without overt cardiovascular disease (CVD)and its predictors. **Methods:** We studied 30 patients with chronic kidney disease stage 3 and 4, who did not have self-reported CVD. Hs-cTnT was measured byusing a highly sensitive assay on an automated Roche/Cobas e411 platform, andwas correlated with demographic, laboratory and echocardiographic features of the studied patients. **Results:** Hs-cTnT was positive in 66.7 % of subjects. Lower eGFR was associated with higher hs-cTnT, patients with stage 4 CKDhad 3-fold higher hs-cTnT compared to subjects with stage 3 CKD. Older age, male gender, LV mass, diabetes, higher blood pressure and the presence of albuminuria wereall positively correlated with hs-cTnT. **Conclusion:** Hs-cTnT was found positively correlated with several cardiovascular risk factors in CKD patients, also it was positively correlated with LVMI. This shows that hs-cTnT is a good marker of cardiovascular risk in CKD patients.

Keywords: High sensitivity cardiac troponin T, chronic kidney disease, cardiovascular disease.

Introduction:

Chronic kidney disease ranges from stage 1, where eGFR ≥ 90 ml/min/1.73m^2 to stage 5, where eGFR< 15 ml/min/1.73m^2 and who may require renal replacement therapy. It is considered a worldwide public health problem with an increasing incidence and prevalence, poor outcomes, and high cost.[1]

Cardiovascular disease is frequently associated with chronic kidney disease, and it is the main cause of death of CKD patients even in early stages. However, several risk factors of cardiovascular disease in chronic kidney disease are treatable.[2]

To identify chronic kidney disease patients with high liability to cardiovascular disease, several biomarkers have been used such as serum albumin, urinary albumin to creatinine ratio, and assessment of left ventricular hypertrophy by using echocardiography.

The high-sensitivity cardiac troponin T assay is a quantitative analysis test of very low levels of troponin T in the blood. The high sensitivity cardiac troponin T assay is being increasingly used as a marker for cardiac risk and as a prognostic tool in heart disease. In addition, it is also used to confirm acute coronary syndrome in chronic kidney disease patients who have a high pretest probability.[3]

Aim of the study:

The aim of the present work was to assess high sensitivity cardiac troponin T as a marker of cardiovascular risk in asymptomatic patients and its correlation with echocardiography findings in chronic kidney disease patients.

Subjects:

This study was conducted on 30 patients with chronic kidney disease stages 3 and 4, who were attending the outpatient clinic or who were admitted into the internal medicine department of the Main University Hospital of Faculty of Medicine, Alexandria University, during the period from July 2015 to December 2015. The exclusion criteria for this study includeda history of ischemic heart disease, acute coronary syndrome, valvular heart disease, multiple myeloma, chemotherapy, immunosuppressive drugs, hemodialysis in the last 6 months, vasculitis, pericarditis and acute myocarditis.

Methods:

All individuals included in this study were subjected to full history taking, full clinical examinations and laboratory investigations including;[4] hemoglobin level, serum uric acid, calcium, phosphorus, alkaline phosphatase, cholesterol, triglycerides, urine albumin to creatinine ratio, and estimated GFR by the abbreviated Modification of Diet in Renal Disease Study (MDRD) formula. High sensitivity cardiac troponin T test was performed on whole blood samples, collected into vacuum tubes with ethylene ediamine tetra acetic acid.[5] It was measured using a highly sensitive assay on an automated Roche/Cobas e411 platform. All patients were subjected to electrocardiography and transthoracic echocardiography,[6] using its m-mode for measurement of: left ventricular end diastolic dimension,left ventricular end systolic dimension, left ventricular ejection fraction (LVEF= $\frac{\mathbf{EDV - ESV}}{\mathbf{KDV}}$ X 100), wall motion abnormalities and left ventricular mass index (LVMI). All data were analyzed using SPSS and significance was considered at the 5% level.[7]

Results:

The present study was carried out on 30 patients presenting with chronic kidney disease stages 3 and 4.The ages of the patients in this study ranged between 21-70 years with a mean of 51.33±13.12 years. Fourteen patients were males (46.7%), while sixteen patients were females (53.3%).Only seven patients were smokers (23.3%).The causes of CKD in the studied patients include diabetic kidney disease in eleven patients (36.7%), and hypertension in ten patients (33.3%). The eGFR by MDRD for the patients ranged between 16.24-43.07 ml/min /1.73 m2 with a mean of 85.15±23.80 ml/min/1.73m2, Seven patients were in stage 3CKD (23.3%) while twenty three patients with stage 4 CKD (76.7%). Three patients of this group had normal to mildly increased albuminuria (<30mg/g; 10%), while seven patients had moderately increased albuminuria (30-299 mg/g; 23.3%), and twenty patients had severely increased albuminuria (> 300 mg /g; 66.7%).

Hs-cTnT of all patients ranged between 4.18 and 142.80, with a mean of 52.48 ± 45.22 and a median of 42.85 mg/dl. The cut-off value of hs-cTnT commonly used is 14 ng/l, so there are twenty patients who tested positive for hs cTnT (66.7%), and ten patients who tested negative for hs cTnT (33.3%).

ECG showed signs of left ventricular hypertrophy in nineteen patients (63.3%). On echocardiography, Twenty two patients had normal ejection fraction (73.3%), three patients had mildly decreased ejection fraction (10%), while three patients had moderately decreased ejection fraction (10%), and two patients had severely decreased ejection fraction (6.7%). Twenty five patients had normal fractional shortening (83.8%), two patients had mildly decreased fractional shortening(6.7%), while one patients had moderately decreased fractional shortening (3.3%), and two patients had severely decreased fractional shortening (6.7%). End diastolic dimension of all patients ranged between 41-69 mm with a mean of

54.17±7.39 mm, twenty patients had normal measures (66.7%), three patients had mildly dilated EDD (10%), while four patients had moderately dilated EDD (13.3%), and three patients had severely dilated EDD (10%). Nineteen patients had normal end systolic dimension ESD (63.3 %), while eleven patients had increased ESD (36.7%). Left ventricular mass of all patients ranged between 135-342 g with a mean of 229.57±59.96 g, fifteen patients had normal LVM (10%), four patients had mildly increased LVM (13.3%), while six patients had moderately increased LVM (20%), and five patients had severely increased LVM (16.7%). Left ventricular mass index of all patients ranged between 63-178g/m2, with a mean of 118.4±31.49 g/m2,eleven patients are within normal range (36.7%), while nineteen patients are abnormally increased (63.3%).

Hs-cTnT was positively correlated with age (r_s =0.530, p =0.003, figure 1), LVMI (r_s = 0.654, p=0.001, figure 2), the presence of D.M (p=0.001), hypertension (p=0.003), and UACR (p=0.030). On the other hand, there was a negative significant correlation between high sensitivity cardiac troponin T with eGFR (r_s= -0.675, p< 0.001, figure 3) and serum albumin (r_s = -0.579, p =0.001, figure 4).

Table (I): Summary of patient's demographic, clinical and laboratory data.

Clinical characteristics	Reference range	Range	Mean and SD
Age (years)	-	21-70	51.33±13.12
Male sex (%)	-	(46.7)	14 patients
Female sex (%)	-	(53.3)	16 patients
Smoker (%)	-	(23.3)	7 patients
Hypertension (%)	-	(33.3)	10 patients
Diabetes (%)	-	(36.7)	11 patients
Cholesterol (mg/dl)	200	114-256	204.83 ± 34.23
Triglycerides (mg/dl)	175	84-269	185.43 ± 43.25
Hemoglobin (g/dl)	12-14	6.9-11.5	6.90-11.50
BMI (kg/ m^2)	18.5-24.9	19.90-38.10	27.34±3.82
Serum albumin (g/dl)	3.5-5.5	1.8-4.3	3.14 ± 0.70
eGFR (ml/min/1.73m^2)	90-120	16.24-43.07	85.15±23.80
Hs-cTnT (ng/l)	14	4.18-142.80	52.48 ± 45.22
UACR (mg/g)	<30	15-2985	1032.81 ± 891.29
LVEF (%)	≥ 55	25-82	57.90 ± 13.51
FS (%)	25-43	12-50	31.73± 9.06
LVMI (g/m^2)	43-115	63-178	118.40±31.49

BMI: Body mass index
eGFR: Estimated glomerular filtration rate
Hs-cTnT: High sensitivity cardiac troponin T
UACR: Urinary albumin to creatinine ratio
LVEF: Left ventricular ejection fraction
FS: Fractional shortening
LVMI: Left ventricular mass index

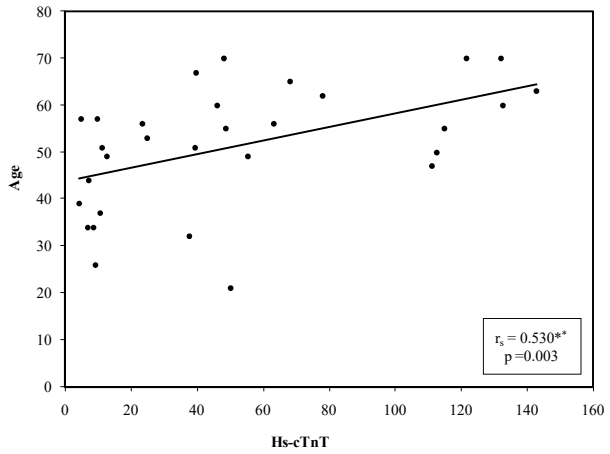

Figure (1): Correlation between Hs-cTnT with age.

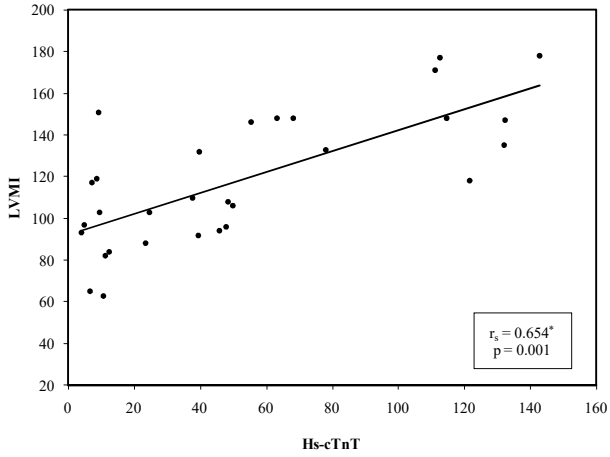

Figure (2): Correlation between Hs-cTnT with LVMI

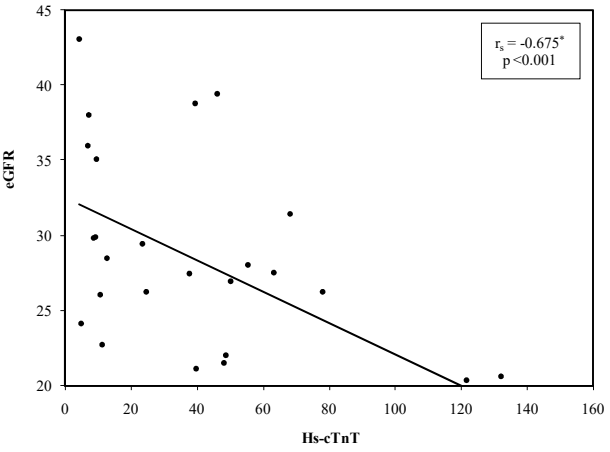

Figure (3): Correlation between hs-cTnT with eGFR

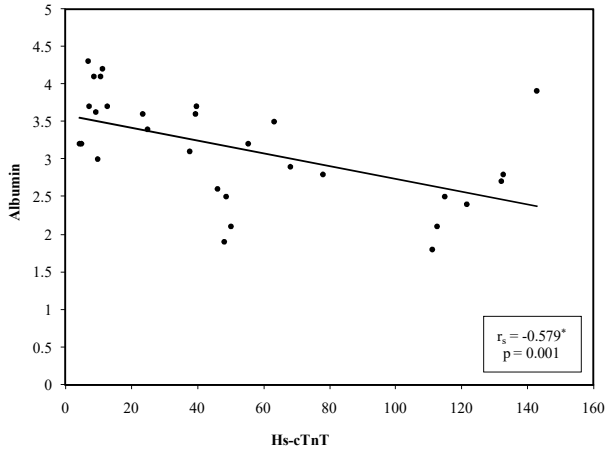

Figure (4): Correlation between Hs-cTnT with serum albumin

Discussion:

Cardiovascular disease is the leading cause of morbidity and mortality in chronic kidney disease patients, occurring even in the earliest stages of chronic kidney disease. High sensitivity cardiac troponin T may be a marker of cardiovascular risk in chronic kidney disease patients.

In our study, we found that there was a statistically significant positive correlation between high sensitivity cardiac troponin T and age (r_s = 0.530, p = 0.003). Similarly, Drazner et al.[8] found that older age was independently associated with elevated high sensitivity cardiac troponin T in CKD patients, this can be explained by microvascular injury changes associated with advancing age.

We found that patients with diabetes mellitus were more likely to have positive hs-cTnT level, (z=3.244, p=0.001), as diabetes mellitus increases progression of atherosclerosis. Also patients with hypertension were more likely to have positive hs-cTnT level, (p=0.001). Similarly Drazner et al, [8] found that diabetes was independently associated with elevated high sensitivity cardiac troponin T. Also Hellemons et al,[9] found that elevated hs-cTnT may reflect blood pressure abnormalities in CKD patients.

In addition, we found that there was a statistically significant positive correlation between urinary albumin to creatinine ratio and high sensitivity cardiac troponin T (p = 0.025). Hemmelgarn et al,[10] also observed an independent association of high sensitivity cardiac troponin T with urinary albumin to creatinine ratio.

Diabetes mellitus, hypertension and urinary albumin creatinine ratio are widely recognized as cardiovascular risk factors in chronic kidney disease patients.[11]

In our study, we found that there was a statistically significant negative correlation between serum albumin and high sensitivity cardiac troponin T (r_s = -0.579) (p = 0.001). Shah et al,[12]also found that hypoalbuminemia is a strong risk factor for cardiovascular disease. This can be explained by the complex syndrome of malnutrition, inflammation and oxidative stress in patients with chronic kidney disease. We also found that there was a statistically significant negative correlation between high sensitivity cardiac troponin T and estimated glomerular filtration rate (r_s=-0.675, p = 0.017), as CKD on its own is a cardiovascular risk factors independent of malnutrition, uremic toxins, and oxidative stress. Mishra et al,[13] also found that patients with chronic kidney disease are associated with increased levels of high sensitivity cardiac troponin T. Di Angelantonio et al,[14]also found that there is agraded increase inhigh sensitivity cardiac troponin T occurs with worsening renal function.

Lastly, we found that there was a statistically significant positive correlation between high sensitivity cardiac troponin T and left ventricular mass index (p = 0.015). Mallamaci *et al*.[15] found a similar association between high sensitivity cardiac troponin T and left ventricular mass index. Aproposed explanation is the presence of systolic dysfunction in predialysis patients leading to uremic cardiac hypertrophy. Myocardial capillary growth in these cases may not keep up with cardiomyocyte hypertrophy, resulting in cardiomyocyte-capillary mismatch, increased oxygen diffusion distance, and reduced ischemic tolerance of the heart.

Thus our study showed that high sensitivity cardiac troponin T is associated with different cardiovascular risk factors on one hand, and with left ventricular mass index, an actual measure of cardiac hypertrophy on the other hand.

In conclusion:

High sensitivity cardiac troponin T is a good diagnostic and prognostic tool of cardiovascular disease risk in chronic kidney disease patients with stage 3 and 4. Further studies are needed to guide therapy and to test the effect of strict control of cardiovascular risk factors in these patients.

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Original Article

Interleukin-33 (IL-33) in Systemic Sclerosis and its Association with Early Cardiovascular Involvement By Using Magnetic Resonance Imaging.

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Abstract:

The aim of this study: was to determine serum levels of IL-33 in systemic sclerosis patients and evaluate its association with early cardiovascular involvement by using magnetic resonance imaging. Group (A): included 30 adult patients with Systemic Sclerosis (SSc), all diagnosed according to the American college of rheumatology (ACR) criteria for SSc. Group (B): included 20 healthy adult persons (age and sex matched) as a control group. **Methods:** Forced vital capacity (FVC) was measured using a PKM spiroflow spirometer, Cardiac magnetic resonance examination by using delayed enhancement technique, Carotid Doppler to evaluate carotid intima-media thickness (IMT) and plaque stiffness. Computed tomography (CT) of the chest and coronary calcium scoring, Serum IL-33 levels were examined by enzyme-linked immunosorbent assay in 30patients with SSc and 20 healthy individuals, Skin assessment using modified Rodnan Skin Score. **Results:** IL-33 was increased in all SSc patients as compared to controls.IL-33 and clinical findings in diffuse subtype dSSc patients had a positive correlation between the presence of joint contracture. There is statistically significant correlation between patients with CMR findings and modified rodnan skin score Increased level of IL-33 ,positive findings in CMR also increased in both diffuse and limited subtypes ,however statistical significance was only with diffuse one with (p=0.031), Higher level if IL-33 (median =100 pg/ml) was associated with positive findings in HRCT in statistically significance ,IL-33 is highly correlated to the presence of pulmonary fibrosis, skin sclerosis, joint contracture and modified rodnan skin score, So IL-33levels were increased in SSc patients and correlated with the extent of skin sclerosis and the severity of pulmonary fibrosis. Therefore, IL-33 possibly plays a role in cutaneous and pulmonary fibrosis in SSc patients. **Conclusion:** Cardiac MRI is an excellent measure for identifying early cardiovascular involvement in order to provide a more accurate management of SSc patients. IL33 most probably has as significant role in the pathogenesis of Systemic Sclerosis. IL-33 serum levels paralleled the severity of the disease subset. Understanding of IL-33 functions is important for development of new therapeutic approaches including IL-33 and IL-33 receptor as a therapeutic target.

Keywords: IL33, Systemic Sclerosis & CVD, MRI

Introduction:

Systemic sclerosis (SSc) is a complex chronic connective tissue disease. It is characterized by chronic multisystem involvement of skin and internal organs. SSc is a connective tissue disease characterized by cutaneous and visceral fibrosis [(1)],and widespread vascular pathology. Theunderlying cause for SSc remains poorly understood, but it is well established that microvascular endothelial cells are the target tissue in this disease. [(2)]

The ACR and the European League Against Rheumatism (EULAR) established a committee to provide a joint proposal for new classification criteria for SSc.[(3)]

The new classification criteria shows that skin thickening of the fingers extending proximal to the meta carpo phalangeal joints is sufficient for the patient to be classified as having SSc; if that is not present, seven additive items apply, with varying weights for each: skin thickening of the fingers, fingertip lesions, telangiectasia,

abnormal nailfold capillaries, interstitial lung disease or pulmonary arterial hypertension, Raynaud's phenomenon, and SSc-related autoantibodies. [4]

The vascular involvement of SSc has been considered to be mainly micro vascular. Macro vascular involvement is considered rare. However, increased prevalence of macro vascular disease has been reported in recent literature.[5]

Coronary artery disease is not uncommon in SSc patients,[5] but its prevalence is similar and not greater to that expected in individuals without SSc. An increased prevalence of distal peripheral artery disease in the digits has been found in several studies. [6-8]

Epidemiology Similar to other connective tissue diseases, SSc is more frequent in women than men, with the most common age of disease onset in the 25 to 50 years range.

Pathophysiology Endothelial alterations may lead to a cascade of stimulatory changes that involve many cells, including fibroblasts, T lymphocytes, macrophages, and mast cells. In turn, the activated cells secrete a variety of substances, including cytokines and their soluble receptors and enzymes and their inhibitors. These substances lead to changes in the extracellular matrix compounds, including fibronectin; proteoglycans; and collagen types I, III, V, and VII. Increased collagen deposition in tissues is a characteristic feature of systemic sclerosis. Increased collagen production or disturbances in its degradation can cause excessive collagen deposition in tissues.[9]

Pathogenesis Activation of T cells is evident in lesional tissues and in peripheral blood, and seems to play a direct role in tissue injury. Vascular injury and activation are the earliest and possibly primary events in the pathogenesis of SSc.[10]

Clinical Features

Skin Manifestations Cutaneous telangiectasia, dilations of dermal blood vessels, occur in limited cutaneous SSc and diffuse cutaneous SSc, but are more extensive in limited cutaneous SSc, particularly the subgroup previously designated as CREST (calcinosis, Raynaud's phenomenon, esophageal involvement, sclerodactyly, and telangiectasia) syndrome.[11]

Gastrointestinal Tract Manifestations Esophageal involvement is frequent with dysmotility and lower esophageal sphincter dysfunction, and consequent gastro esophageal reflux is almost universal in SSc.[12]

Musculoskeletal Involvement The fibrotic process of SSc commonly affects the tendons (causing tendon friction rubs), ligaments, and joint capsules, restricting movement.

Contraction of the fingers is a hallmark of SSc, may develop rapidly, and has a significant impact on hand function.[13]

Pulmonary Involvement The most common forms of interstitial lung disease in SSc are histologically classified as usual interstitial pneumonia and nonspecific interstitial pneumonitis.

PAH, defined as an elevation in the mean pulmonary artery pressure greater than 25 mm Hg at rest, occurs in limited and diffuse cutaneous forms of SSc and is a leading cause of mortality.[14]

Renal Manifestations Scleroderma renal crisis occurs in 10% to 15% of patients with diffuse cutaneous SSc and only vary rarely (1% to 2%) in limited cutaneous SSc.[15]

Neurologic Manifestations In early-stage diffuse cutaneous SSc, patients commonly report symptoms of median nerve compression, and many patients undergo surgical treatment for carpal tunnel syndrome before the diagnosis of SSc is established.[15]

Interleukin 33 IL-33 is a newly reported cytokine of IL-1 family. Recent evidence suggests a role for IL-33/ST2 in several rheumatological diseases, including systemic sclerosis, rheumatoid arthritis (RA), osteoarthritis (OA), psoriatic arthritis (PsA) and systemic lupus erythematosus (SLE).[16,17] In SSc, the combination of vascular abnormalities, collagen deposition, and autoimmunity leads to widespread tissue and organ fibrosis.[18] It has been found that, compared to healthy controls, IL-33 expression was significantly increased in SSc patients. Meanwhile, the serum level of IL-33 was correlated with

early disease stage and micro vascular involvement.[19] Moreover, some other investigators reported the same observations recently.[20] These data prompted us that IL-33 should be involved in the SSc pathogenesis, and the mechanism may be correlated with the role of IL-33 in promoting fibrosis.[21]

Subjects:

The study included 2 groups:

Group (A): included 30 adult patients with Systemic Sclerosis (SSc), all diagnosed according to the American college of rheumatology (ACR) criteria for SSc.[3]

Group (B): included 20 healthy adult persons (age and sex matched) as a control group.

All patients were selected from Internal medicine department, Rheumatology unit, Main Alexandria University hospital.

An informed consent was taken from all patients before the beginning of the study.

Methods:

This study was conducted on 30 patients with Systemic Sclerosis and 15 healthy adult persons as a control group admitted to rheumatology unit at Alexandria Main University Hospital.

The study population was subjected to:

A. Complete History taking including:

- Demographic data.
- History of the present complaints.

B. Thorough clinical examination included the following

- Vital signs
- Head and neck examination
- Cardiovascular examination
- Chest examination
- Abdominal examination
- Skin and extremities

C. Skin assessment using modified Rodnan Skin Score:[22]

Using a semi-quantitative estimation of the degree of skin thickening.

D. Laboratory investigations including:

1- Complete Blood Count, routine blood chemistry.

2- Urine analysis.

3- Anti Scl-70.

4- Anti-centromere.

5- Determination of Serum levels of IL-33 measured by human ELISA.

6- Computed tomography(CT) of the chest and coronary calcium scoring

7- Carotid Doppler: to evaluate carotid intima – media thickness

8- Cardiac magnetic resonance examination to evaluate cardiac anatomy, myocardial perfusion and viability

9- Pulmonary function test using spirometer

RESULTS:

Table (I) shows IL-33 in the studied groups. IL-33 in dSSc patients ranged 80-140 pg/ml with mean value 97.21±13.43 pg/ml, in lSSc ranged 88-125 pg/ml with mean value 103.33±15.56 pg/ml and in control persons ranged 50-84pg/ml with mean value 66.20±10.72 pg/ml values. There was statistical significant difference regarding IL-33 in studied groups (P =0.001). Serum levels of IL-33 were significantly higher in SSc patients than in healthy controls with no significant difference between diffuse and limited subtype regarding serum levels of IL-33 (p=0.363) table (I)

There is statistically significant correlation between patients with CMR findings and modified rodnan skin score with median 30.0 versus 23.0 in patients without CMR CHANGES, So CMR changes correlate significantly with severity of mRSS in patients subtypes.

Table (III**) shows:** With Increased level of IL-33, positive findings in CMR also increased in both diffuse and limited subtypes ,however statistical significance was only with diffuse one with (p=0.031)

Table (IV): shows Higher level if IL-33 (median =100 pg/ml) was associated with positive findings in HRCT in statistically significance with (p=0.031) versus level of IL-33 (median =90 pg/ml) with normal HRCT imaging.

Table (I): Comparison between the studied groups according to readings of IL-33.

	Cases			Control (n=20)	^{KW}p
	Total (n=30)	Diffuse type (n=24)	Limited type (n=6)		
IL-33 pg/ml					
Min. – Max.	80.0 – 140.0	80.0 – 140.0	88.0 – 125.0	50.0 – 84.0	
Mean ± SD.	98.43 ± 13.82	97.21 ± 13.43	103.33 ± 15.56	66.20 ± 10.72	<0.001*
Median	96.0	95.0	98.50	66.0	
Sig.bet.Grps	p_1<0.001*,p_2<0.001*, p_3<0.001*, p_4=0.362				

Table (II**)**: Relation between cardiac MRI findings and modified rodnan skin score in total sample (n=30)

	Cardiac MRI findings		T	P
	No (n =14)	Yes (n =16)		
Modified rodnan skin score				
Min. – Max.	18.0 – 29.0	22.0 – 35.0		
Mean ±SD.	23.64 ± 3.52	29.88 ± 3.81	4.629*	<0.001*
Median	23.0	30.0		

Table (III): Relation between cardiac MRI findings and IL.33 levelin SSc.

Cardiac MRI Findings	N	IL.33 level			Z	P
		Min. – Max.	Mean ± SD.	Median		
Diffuse type						
No	**10**	80.0 – 98.0	90.70 ± 5.98	91.0	2.180*	0.029*
Yes	**14**	85.0 – 140.0	100.79 ± 14.1	100.0		
Limited type						
No	**4**	88.0 - 95.0	92.0 ± 3.56	92.50	1.879	0.060
Yes	**2**	100.0 – 125.0	112.50 ± 17.7	112.50		

Table (IV): Relation between HRCT findings and IL33 level in SSc patients

	HRCT findings		Z	P
	absent (n =11)	present (n =19)		
Il.33 level				
Min. – Max.	80. – 125.0	90.0 – 140.0		
Mean ± SD.	95.16 ± 12.70	104.09 ± 14.42	2.163*	0.041*
Median	90.0	100.0		

Discussion:

One of the proinflamatory cytokines believed to be involved in the pathology of SSC is IL-33.

Recent evidence suggests a role for IL-33/ST2 in several rheumatological diseases, including systemic sclerosis, rheumatoid arthritis (RA), osteoarthritis (OA), psoriatic arthritis (PsA) and systemic lupus erythematosus. (SLE). The present study was conducted on 30 patients with systemic sclerosis fulfilling the ACR criteria for diagnosis of the disease and 15 age- sex matched healthy individual control group.

IL-33 in patients Group A ranged 75-220, Group B ranged 62-98 and in control group ranged 50-84. There was statistical significant difference regarding IL-33 in three studied groups ($P < 0.5$).

In the present study IL-33 was increased in all SSc patients as compared to controls.

Furthermore the levels of IL-33 were significantly higher in the dSSc subset compared to the lSSc subset. Thus IL-33 serum levels paralleled the severity of the disease subset. Similar results have been reported by several studies including Wagner A, et al in 2015.[23] Wagner A, et al suggested that an endothelial, T cell and fibroplast activation can be present in patients with early SSc, suggesting that new routes of investigation of cell-cell dynamics in target tissues predating overt disease manifestations, thus opening new therapeutic approaches.

Furthermore YanabaK, et al in 2011[24] also reported that Serum IL-33 levels were elevated in SSc patients compared with healthy individuals. Patients with diffuse cutaneous SSc had higher levels of IL-33 than those with limited cutaneous SSc.

As in our study, patients with diffuse SSc had higher levels of IL-33 than those with limited cutaneous SSc.

We also studied the correlation of IL-33 levels in SSc patients with clinical manifestations.

We found a significant positive correlation between IL-33 levels and the presence of pulmonary fibrosis, skin sclerosis, Raynaud's phenomenon, pitting scares and ulcers, pulmonary hypertension, Joint contracture and modified rodnan skin score.

Similar results are reported by YanabaK, et al in 2011[24] who reported that IL-33 levels corrected positively with extent of skin fibrosis and pulmonary fibrosis.

Thus suggesting that IL-33 may possibly play a role in organ fibrosis in SSc patients.

Conclusion:

IL-33 was increased in all SSc patients as compared to controls. So we can conclude that IL33 most probably has as significant role in the pathogenesis of Systemic Sclerosis. Furthermore the levels of IL-33 were significantly higher in the dSSc subset compared to the lSSc subset.

Thus IL-33 serum levels paralleled the severity of the disease subset.

IL-33 is highly correlated to the presence of pulmonary fibrosis,skin sclerosis, Raynaud's phenomenon, pitting scares and ulcers, pulmonary hypertension, Joint contracture and modified rodnan skin score.

So understanding of IL-33 functions is important for development of new therapeutic approaches including IL-33 and IL-33 receptor as a therapeutic target.

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Original Article

Effect of Interferon on Prevalence of Insomnia in Chronic Hepatitis C Patients.

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Abstract:

Objective: The goal of this study was to estimate the impact of treatment response on prevalence of insomnia in chronic hepatitis C patients. **Methods:** This is a survey performed to hepatitis C patients using a representative sample of 354 patients aged 20 years or older from Mansoura specialized hospital and from Mansoura general hospital at start and end of treatment with interferon, laboratory and radiological assessment were done, Interviews were conducted using the Berlin insomnia scale. The questions were related to the risk of having sleep apnoea. **Results:** 18.67% (66 patients) of the sample reported insomnia during the study, 28.8% (102 patients) had insomnia both at the start and at the end, so 47.5% (168 patients) had insomnia total, the prevalence was higher in females than in males at start 53.9% vs. 46.1% (55 vs. 47 patients) and increased with age but at end males were more and no effect for age. **Conclusion:** Insomnia is frequent in hepatitis C patients, affecting 47.5% of patients. Results showed that insomnia is differently affected by sex with treatment.

Key Words: Insomnia and hepatitis C.

Introduction:

Hepatitis C Virus (HCV) infection is a major health problem in Egypt, where the prevalence is (10–20) folds higher than that in the USA. Egypt has the highest prevalence of HCV worldwide, ranging from 6% to more than 40% across regions and demographic groups.(1) Insomnia has been studied both as a symptom and as a sign.(2) Insomnia is studied in a variety of ways in the medical literature and popular press. Insomnia is defined by the presence of an individual's report of difficulty with sleep.(3) Insomnia is common and includes symptoms such as difficulty falling asleep or staying asleep, early morning wakening, and sleep dissatisfaction, as well as daytime consequences such as tiredness.(4) Several risk factors for insomnia were reported by the State-of-the-Science conference in June 2005, age and gender are the most clearly identified demographic risk factors, with an increased prevalence in women and older adults. The cause of this increased risk in the elderly is not well defined, it may be due to the partial decline in functionality of sleep control systems that may contribute to insomnia in this older population, the presence of comorbid medical conditions is also a significant contributor to the increased prevalence of insomnia in the elderly, in females insomnia is more prevalent with both the onset of menses and menopause. (5)Many studies investigating prevalence of insomnia in the USA and Europe, including the UK, however less is known about the onset and natural history of insomnia. These studies have highlighted factors associated with prevalent insomnia, and it is not clear what is cause and effect or whether the factors are linked with the onset or persistence of the problem. (6) 60% of chronic untreated HCV patients had sleep problems

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and 30% of patients on interferon therapy also had sleep problems, the non-pharmacological treatment of it includes:

1. Go to sleep when you are sleepy .If you cannot fall asleep within 20 minutes, get up and do something boring until you feel sleepy.
2. Naps as your doctor advises.
3. Have a regular sleep-wake schedule even on weekends.
4. Do regular exercise four hours before bedtime at least.
5. Innovate sleep rituals (listening to music, etc.) as cues to your body to sleep.
6. Use your bed for sleeping and intimacy only.
7. Stop caffeine containing beverages foods and medications nicotine and alcohol at least 4-6 hours before bedtime.
8. Eat a light snack before bed with a glass of milk, which contains sleep promoting tryptophan.
9. Have a hot bath before bedtime, as a drop in the body temperature will promote sleep.
10. Keep your bed and bedroom quiet and comfortable for example a cooler room is recommended and you can use a humidifier if the air is too dry.

The pharmacological treatment includes:

1. Benzodiazepines but has risks like tolerance, dependence, withdrawal, impaired cognition, respiratory depression and disrupted sleep: reduced slow wave sleep and rapid eye movement, it may give short term benefit in the acute phase of treatment of comorbid anxiety or mood disorders, in HCV patients lorazepam, oxazepam and temazepam are preferred as eliminated by glucoronidation which is spared in liver disease. Lorazepam and temazepam are better in maintenance insomnia as they have intermediate half-life.
2. Second generation antipsychotics for a variety of psychiatric disorders, it may be tempting to use sedating antipsychotics for treating primary insomnia but the metabolic risks included: weight gain, diabetes and hyperlipidemia also its great effects on sustained virological response rates during IFN and ribavirin combination therapy. [7]

Aim of this study:

The aim of this study was to study the effect of interferon on prevalence of insomnia in chronic hepatitis C patients.

Patients and Methods:

Study design: Cohort study.

Settings: This study was carried out at the Liver Unit of Mansoura University Hospital and Mansoura General Hospital.

Sample and sampling technique:

The study was carried out on 354 adult patients with chronic hepatitis C, of them 258 were males (72.88%) and 96 were females (27.11%). Informed consent to be included into the study was obtained from each patient.

Inclusion criteria: age > 20 years, Positive HCV antibody and HCV- RNA, Patients with liver biopsy proven chronic hepatitis, HBsAg negative.

Exclusion criteria: Age<20 years, co-infection with HBV, active alcohol consumption, HBsAg positive, pre-existing psychiatric condition, pregnancy or breast feeding and co-morbidities.

All patients have been subjected to thorough history taking and complete clinical examination, Laboratory investigations including: CBC (WBCs, hemoglobin & platelets), Bleeding time (BT), Alanine amino transferase (ALT) (U/L), Aspartate amino transferase (AST) (U/L), Serum bilirubin (mg/dl), Serum albumin (g /dl), Alkaline phosphatase (U/L), International normalized ratio (INR), Viral markers including HCV- antibody, HCV- RNA (IU/m1) and Hepatitis B surface antigen, ANA titre, SMA, AMA and LKM, TSH level, α fetoprotein (ng/dL), and pregnancy test for females**.**

Data collection and ethical considerations:

Consent to participate was implied by the return of the completed Berlin questionnaire[8], it was performed in the period between April 2012 and April 2013. completed by respondents on a voluntary choice and they were assured of confidentiality of their response, it was done for all patients after translation to Arabic and replied either by the patient directly or his relative asked him if he can't read, patients answered 3 groups of questions related to the risk of having sleep

apnoea, patient was considered low risk if one or no categories with positive score and he was considered high risk if two or more categories with positive score. The questionnaire includes: height (m), weight (Kg), age, sex and request to choose the correct answer

Group 1:

1. Do you snore?

a. yes

b.no

c. don't know

If you snore:

2. How your snoring is?

a. slightly louder than breathing.

b. as loud as talking

c. louder than talking.

d. very loud-can be heard in adjacent rooms.

3. How often do you snore?

a. nearly every day.

b. 3-4 times a week.

c. 1-2 times a week.

d. 1-2 times a month.

e. never or nearly never.

4. Has your snoring ever bothered other people?

a.yes

b.no

c.don't know

5. Has anyone noticed that you quit breathing during your sleep?

a.nearly every day.

b.3-4 times a week.

c.1-2 times a week.

d.1-2 times a month.

e.never or nearly never.

Group 2:

6. How often do you feel tired or fatigued after your sleep?

a. nearly every day.

b. 3-4 times a week.

c. 1-2 times a week.

d. 1-2 times a month.

e. never or nearly never.

7. During your waking time, do you feel tired, fatigued or not up to par?

a. nearly every day.

b. 3-4 times a week.

c. 1-2 times a week.

d. 1-2 times a month.

e.never or nearly never

8. Have you ever nodded off or fallen asleep while driving a vehicle?

a. yes

b. no

if yes:

9. How often does this occur?

a. nearly every day.

b. 3-4 times a week.

c. 1-2 times a week.

d. 1-2 times a month.

e. never or nearly never

Group 3:

10. Do you have high blood pressure?

a.yes

b. no

c.don't know

Group 1:

Question number 1: if yes 1point

Question number 2: if c or d 1point

Question number 3: if a or b 1 point

Question number 4: if a 1 point

Question number 5: if a or b 2 points, add points

Group 1 is positive if total score is 2 or more points.

Group 2:

Question number 6: question number 3: if a or b 1 point

Question number 7: if a or b 1 point

Question number 8: if a 1 point

Question number 9: noted separately, add points

Group 2 is positive if total score is 2 or more points.

Group 3: is positive if question number 10 is yes or the BMI is greater than 30/m^2

Statistical analysis:

Collected data were entered and analyzed using SPSS software version 17 qualitative data were expressed as count and percent and compared with Chi square or Fisher exact test quantitative data were expressed as Mean ±Standard deviation (SD) or Median and compared using Independent sample T test if normally distributed or Mann-Whitney test if not.

Results:

As shown in table (I) 18.67% (66 patients) had insomnia during the study, 28.81% (102 patients) had insomnia both at the start and at the end, 47.5% (168:66&102) had insomnia total, 52.5 %(186 patients) had no insomnia all through, the prevalence was higher in females than in males at start 53.9% vs. 46.1% (55 vs 47) but at end males were more 81.8%vs18.2%(54 vs 12)

As shown in table (II) there is significant increase of insomnia with age at start of treatment.

As shown in table (3) there is no significant changes of insomnia with age at end of treatment.

Table (I): Insomnia Groups:

	No Insomnia althrough	Insomnia althrough	No Insomnia at start but develops on treatment	Total	X^2	P value
Male	84.4% 157	46.1% 47	81.8% 54	258	52.247	<0.0001
Female	15.6% 29	53.9% 55	18.2% 12	96		
Total	52.5% 186	28.81% 102	18.67% 66	354		

Table (II): Age versus Insomnia groups before starting therapy

	No significant sleep disorder	Significant sleep disorder	Total	X^2	P value
Age between 20 and 30	30	0	30	17.74	<0.0001
Age between 30 and 40	93	33	126		
Age between 40 and 50	105	51	156		
Age between 50 and 6	24	18	42		
Total	252	102	354		

Table (III): Age versus Insomnia groups at end of therapy

	Age between 20 and 30	Age between 30 and 40	Age between 40 and 50	Age between 50 and 60	Total	X^2	P value
No significant sleep disorder	18	69	81	18	186	2.52	<0.47
Significant sleep disorder	12	57	75	24	168		
Total	30	126	156	42	354		

Discussion:

Insomnia needs to be assessed as such because it is multidimensional.[4] Patients with insomnia had greater loss of function than patients with congestive heart failure in reported pain, emotional effects, and mental health effects. Also, those patients reported more physical problems than patients with depression. During the daytime consequences of insomnia, the increased occurrence of accidents poses the greatest health risk. Insomniacs are 2.5 to 4.5 times more likely than controls to have an accident .The most common complications associated with insomnia are psychiatric disorders. It is estimated that 40% of all insomnia patients have a coexisting psychiatric condition. Among these psychiatric disorders, depression is the most common, heart rates were increased and variability was decreased in all stages of sleep in insomnia patients compared to healthy normal sleepers.[2]

Our study showed that 47.5% of patients had insomnia: 18.67% (66 patients) had insomnia during the study, 28.81% (102 patients) had insomnia both at the start and at the end, 52.5% (186 patients) had no insomnia all through, consistent with Roth who stated that insomnia is widely prevalent and affects approximately 30% of the population.[2]

The study showed that 18.67% (66 patients) had insomnia during the study, 28.81% (102 patients) had insomnia both at the start and at the end, 52.5% (186 patients) had no insomnia all through, The prevalence was higher in females than in males at start 53.9% vs. 46.1% (55 vs 47) but at end males were more 81.8% vs 18.2% (54 vs 12) which is consistent with Ohayon and Sagales[4] who stated that the prevalence was higher in females than in males (23.9% vs. 17.6%).

And also we found that insomnia increased with age which is consistent with Weyerer, Klink and Ohayon[9,10,11] who found a clear relationship between increasing age and insomnia.

At end of study we found that males had more insomnia than females which may be either due to good response of female to treatment or due to more stresses males are exposed to.

Conclusion:

Insomnia is frequent in hepatitis C patients, affecting up to half individuals (47.5%). Results showed that insomnia is differently affected by sex with treatment.

Recommendations:

More wide scale studies are needed to explain the insomnia in HCV patients

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Original Article

The Relation between the Level of Serum Transforming Growth Factor Beta and Hemodialysis Adequacy in Diabetic and Non Diabetic Patients on Maintenance Hemodialysis.

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Abstract:

Background: Hemodialysis is still the most common renal replacement therapy (RRT) modality in end stage renal disease patients (ESRD), the first problem to be faced when choosing hemodialysis for patients with ESRD is the vascular access, dialysis delivery should be adequate not only to improve quality of life but also to prolong survival, quality of life adjusted for life expectancy defined kt/v of 1.3 as the optimal cost-effective dialysis, An ideal access delivers a flow rate to the dialyzer adequate for the dialysis prescription, has a long use-life, and has a low rate of complications (eg, infection, stenosis, thrombosis, aneurysm, and limb ischemia). Of available accesses, the surgically created fistula comes closest to fulfilling these criteria, working fistula must have all the following characteristics; blood flow adequate to support dialysis which usually equates to blood flow greater than 600 ml/min, a diameter greater than 0.6 cm, with a location accessible for cannulation and a depth of approximately 0.6 cm (ideally between 0.5 and 1cm) from the skin surface. In hemodialysis patients with an arteriovenous fistula (AVF), access failure is primarily due to fistula stenosis, which predisposes to thrombosis and subsequent access loss. The risk for access failure differs individually, Fistula stenosis is histologically characterized by intimal hyperplasia, which is induced by growth factors, among which transforming growth factor β1 (TGF-β1) is of major importance. TGF-β1 influences the risk for hemodialysis access failure. By inducing synthesis of extracellular matrix proteins, overproduction of TGF-β1 may accelerate the development of intimal hyperplasia, resulting in fistula stenosis and subsequent access failure, In diabetic ESRD patients there is advanced calcified atherosclerosis which leads to frequently inadequate arterial inflow and eventually also to venous run-off problems. So ESRD patients with diabetes have worse access survival rates and hemodialysis adequacy. **Methods:** the study was conducted to 60 ESRD patients divided to group I (30 Diabetic ESRD patients on HD) and group II (30 Non - diabetic ESRD patients on HD). We estimate serum TGF-beta 1 in all patients. Assess AVF by Doppler U/S and estimate hemodialysis adequacy by using single pool Kt/v **Results:** we found that serum TGF-beta 1 level is significantly elevated in diabetic group, Kt/v is significantly decreased in diabetic group, AVF vein diameter was statistically significantly decreased in diabetic group, we also found that TGF beta 1 was statically positively significant with duration of dialysis, FBG and cholesterol level, lastly we found that TGF-beta is significantly correlated with Kt/v and may affect hemodialysis adequacy adversely particularly in diabetic ESRD patients on HD **Conclusion:** TGF-beta is significantly elevated in diabetic ESRD patients on HD, negatively correlated with AVF vein diameter and significantly correlated with Kt/v and may affect hemodialysis adequacy adversely particularly in diabetic patients on HD.

Keywords: ESRD, Hemodialysis, arteriovenous fistula, TGF-beta 1, Hemodialysis adequacy, diabetics.

Introduction:

Hemodialysis is still the most common renal replacement therapy (RRT) modality in end stage renal disease patients (ESRD). The first problem to be faced when choosing hemodialysis for patients with ESRD is the vascular access, In diabetic ESRD patients there is advanced calcified atherosclerosis which leads to frequently inadequate arterial inflow and eventually also to venous run-off problems. So ESRD patients with diabetes

have worse access survival rates and hemodialysis adequacy. [1]

Dialysis delivery should be adequate not only to improve quality of life but also to prolong survival.[2] The aim of dialysis is thus, to decrease morbidity, increase quality of life and prolong life span.[2] To achieve this dialysis must be performed effectively. [3] Inadequate dose of dialysis increases duration of hospitalization and the overall cost of care. [4]

One method of assessing dialysis adequacy is calculation of kt/v. This index reflects the efficiency of dialysis and correlates with mortality and morbidity rate of patients. Quality of life adjusted for life expectancy defined kt/v of 1.3 as the optimal cost-effective dialysis dose.[4]

Vascular access is vital to delivering adequate hemodialysis therapy. The type of vascular access used in HD patients is recognized to have a significant influence on survival. The use of a tunneled cuffed catheter (TCC) is associated with a substantially greater risk of sepsis, hospitalization and mortality compared to the use of AVF. [5-8]

An ideal access delivers a flow rate to the dialyzer adequate for the dialysis prescription, has a long use-life, and has a low rate of complications (eg, infection, stenosis, thrombosis, aneurysm, and limb ischemia). Of available accesses, the surgically created fistula comes closest to fulfilling these criteria.[9,10]

The National kidney Foundation (NKF) issued the Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines for Vascular Access in an effort to improve patient survival and quality of life, reduce morbidity, and increase efficiency of care.[9]

Two primary goals were originally put forth in vascular access guidelines:

- Increase the placement of native fistulae.
- Detect access dysfunction before access thrombosis.[9]

In general, a working fistula must have all the following characteristics; blood flow adequate to support dialysis which usually equates to blood flow greater than 600 ml/min, a diameter greater than 0.6 cm, with a location accessiblefor cannulation and a depth of approximately 0.6 cm (ideally between 0.5 and 1cm) from the skin surface.[9]

Access stenosis or thrombosis is a costly threat to patency in association with significant morbidity to the patient. Native fistula patency is significantly better than synthetic grafts and should be considered as the first method in maintaining long-term vascular access patency.[11,12]

Studies investigating the pathophysiology of vascular access stenosis which predisposes to thrombosis suggest that the endothelial repair response to injury in the face of excess growth promoters, inflammation and oxidative stress leads to luminal hyperplastic intimal growth. In the presence of prothrombotic environment in the renal patient, vascular thrombosis can occur. The typical lesion of access thrombosis is new intimal vascular smooth muscle cell proliferation in the anastomotic draining vein, this can occur in response to endothelial injury due to repeated vein cannulation. Approximately 50-70% of lesions are within 3-5 cm of the vein anastomosis.[13]

In hemodialysis patients with an arteriovenous (AV) fistula, access failure is primarily due to fistula stenosis, which predisposes to thrombosis and subsequent access loss. The risk for access failure differs individually, an observation that is independent from vascular anatomy in a significant number of patients. Fistula stenosis is histologically characterized by intimal hyperplasia, which is induced by growth factors, among which transforming growth factor β1 (TGF-β1) is of major importance.[14,15]

TGF-β1 influences the risk for hemodialysis access failure. By inducing synthesis of extracellular matrix proteins, overproduction of TGF-β1 may accelerate the development of intimal hyperplasia, resulting in fistula stenosis and subsequent access failure. [15]

A haphazard hyperplastic smooth muscle response of the intima with angiogenesis occurring in both intima and adventitia is associated with the presence of macrophages and cytokines, including transforming growth factor beta1 (TGF- β1), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF).[15]

The pathologic features of HD vascular access stenosis are composed of intimal hyperplasia, VSMC proliferation in the media with subsequent migration to intima, and excessive accumulation of extracellular matrix, which are mediated by several growth factors among which TGF-β and PDGF are of major importance. (16)

Vascular access dysfunction is a well-known cause for a reduction in delivered dialysis, although the prevalence of this problem as a cause for a fall in Kt/v is not known, Inadequate vascular access flow rate due to stenosis leads to mixing of blood from the venous side of the dialysis circuit into the arterial inflow line. This reduces the concentration gradient and reduces net removal for dialyzable solutes. (17)

Aim of the Study:

The aim of this work is to study the relation between serum transforming growth factor beta 1 (TGF-β 1) and hemodialysis adequacy in diabetic and non-diabetic ESRD patients on maintenance hemodialysis by early detection of AVF dysfunction.

Methods & Subjects:

The study will be conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and informed consent will be obtained from each patient. The study was carried out in Dialysis units in Armed Forces Hospital & Police Hospital, Alexandria on 60 elderly ESRD patients on HD & 15 healthy elderly as a control.

Our subjects were divided into 3 main groups with 4 subgroups:

Group I : 30 diabetic ESRD patients on HD

Group II: 30 non-diabetic ESRD patients on HD

Group III: 15 healthy controls.

Exclusion criteria:

1. Patients with less than 3 months duration of the native arterivenous fistula.
2. Patients with hypotension, systemic infection within one month before entry in the study or on warfarin therapy.
3. Known chronic inflammatory disease other than chronic kidney disease (CKD) as systemic lupus erythromatosis (SLE) and vasculitis.
4. Smoking.

All patients will be subjected to the following:

- Full history talking
- Routine investigations: Complete blood picture, Triglycerides (TG), total cholesterol, low density lipoprotein (LDL) and high density lipoprotein (HDL), Fasting blood glucose level, serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), prothrombin time and activity, total proteins, serum albumin), Serum Calcium, serum phosphate.
- TGF- β1 level measurement with enzyme linked immunosorbent assay (ELISA) technique.
- Doppler US study of the native AVF.
- Hemodialysis adequacy. (Kt/v).

Results:

Our subjects were divided into 3 main groups with 4 subgroups:

Group I: 30 diabetic ESRD patients on HD divided to 2 subgroups:

Group Ia: 15 diabetic ESRD patients on HD with functioning AVF between 3 months and 6 months.

Group Ib: 15 diabetic ESRD patients on HD with functioning AVF more than one year.

Group II: 30 non-diabetic ESRD patients on HD divided to 2 subgroups:

Group IIa: 15 non- diabetic ESRD patients on HD with functioning AVF between 3 months and 6 months.

Group IIb: 15 non-diabetics ESRD patients on HD with functioning AVF more than one year.

Group III: 15 healthy controls.

Serum TGF beta: Table (I)

- In group I, the serum TGF beta ranged from 7985.5 pg/ml to 39222.5 pg/ml with a mean of 23450.6 ± 8265.5 pg /ml.
- In group II, the serum TGF beta ranged from 6676.3 pg/ml to 30088.7 pg/ml with a mean of 15785.3 ± 7200.6 pg/ml..
- In group III (Healthy Control), the serum TGF beta ranged from 4790.4 pg/ml to 230896 pg/ml with a mean of 8813.2 ± 5757.3pg/ml.
- Serum TGF beta was statistical significant between 3 groups being higher in group I as compared to group II as compared to group III (Healthy control)

Ultra Sound Doppler study of arterio venous fistula: Table (II)

1- Central venous system stenosis:

- In group I, there were 9 patients with central venous system stenosis (30%)
- In group II, there were 6 patients with central venous system stenosis (20%).
- No significant statistical difference was observed between the two groups regarding the Central venous system stenosis.

2- Venous Thrombosis:

- In group I, there was 1 patient with venous thrombosis (3.3%)
- In group II, there were 5 patients with venous thrombosis (16.7%).
- No significant statistical difference was observed between the two groups regarding the venous thrombosis.

3- Aneurysmal dilatation:

- In group I, there were 3 patients with aneurysmal dilatation (10%)
- In group II, there were 4 patients with aneurysmal dilatation (13.3%).
- No significant statistical difference was observed between the two groups regarding the aneurysmal dilatation.

4- Volume:

- In group I, the volume ranged from 350 ml to 1400 ml with a mean of 708.33 ± 272.63
- In group II, the volume ranged from 500 ml to 1400 ml with a mean of 825.0 – 276.91
- No significant statistical difference was observed between the two groups regarding the AVF volume.

5- Vein diameter:

- In group I, the vein diameter ranged from 0.4 cm to 0.8 cm with a mean of 0.61 ± 0.11
- In group II, the vein diameter ranged from 0.4 cm to 0.9 cm with a mean of 0.69 ± 0.11
- Vein diameter was statistical significant between 2 groups being higher in group II as compared to group I.

6- Osteum diameter:

- In group I, the osteum diameter ranged from 0.5 cm to 0.9 cm with a mean of 0.77 ± 0.11
- In group II, the osteum diameter ranged from 0.5 cm to 1 cm with a mean of 0.72 ± 0.14
- No significant statistical difference was observed between the two groups regarding theOsteum diameter.

Ultra Sound Doppler study of arterio venous fistula: Table (VI)

1- Central venous system stenosis:

- In group Ia, there were 4 patients with central venous system stenosis (26.7%)
- In group Ib, there were 5 patients with central venous system stenosis (33.3%).
- In group IIa there were 4 patients with central venous system stenosis (26.7%)
- Group IIb there were 4 patients with central venous system stenosis (26.7%)
- No significant statistical difference was observed between the four groups regarding the central venous system stenosis.

2- Venous Thrombosis:

- In group Ia, there was 0 patient with venous thrombosis (0%)
- In group Ib, there were 1 patients with venous thrombosis (6.7%).
- In group IIa there were 1 patients with venous thrombosis (6.7%)
- In Group IIb there were 4 patients with venous thrombosis (26.7%)
- No significant statistical difference was observed between the four groups regarding the venous thrombosis.

3- Aneurysmal dilatation:

- In group Ia, there was 1 patient with aneurysmal dilatation (6.7%)
- In group Ib, there were 2 patients with aneurysmal dilatation (13.4%).
- In group IIa there were 0 patients with aneurysmal dilatation (0%)

- In group IIb there were 4 patients with aneurysmal dilatation (26.7%)
- No significant statistical difference was observed between the four groups regarding the aneurysmal dilatation.

4- Volume:

- In group Ia, the volume ranged from 350 ml to 1300 ml with a mean of 753.33 ± 286.90
- In group Ib, the volume ranged from 400 ml to 1400 ml with a mean of 663.33 ± 259.44
- In group IIa, the volume ranged from 500 ml to 1300 ml with a mean of 823.33 ± 235.94
- In group IIb, the volume ranged from 500 ml to 1400 ml with a mean of 826.67 ± 321.20
- No significant statistical difference was observed between the four groups regarding the volume.

5- Vein diameter:

- In group Ia, the vein diameter ranged from 0.4 cm to 0.8 cm with a mean of 0.63 ± 0.11
- In group Ib, the vein diameter ranged from 0.4 cm to 0.8 cm with a mean of 0.59 ± 0.11
- In group IIa, the vein diameter ranged from 0.4 cm to 0.9 cm with a mean of 0.70 ± 0.14
- In group IIb, the vein diameter ranged from 0.5 cm to 0.9 cm with a mean of 0.69 ± 0.08
- No significant statistical difference was observed between the four groups regarding the vein diameter.

6- Osteum diameter:

- In group Ia, the osteum diameter ranged from 0.5 cm to 0.9 cm with a mean of 0.77 ± 0.12
- In group Ib, the osteum diameter ranged from 0.6 cm to 0.9 cm with a mean of 0.76 ± 0.11
- In group IIa, the osteum diameter ranged from 0.5 cm to 0.9 cm with a mean of 0.70 ± 0.12
- In group IIb, the osteum diameter ranged from 0.5 cm to 1 cm with a mean of 0.74 ± 0.15
- No significant statistical difference was observed between the four groups regarding the Osteum diameter.

Kt/v: Table (V)

- In group I, the Kt/v ranged from 0.87 to 1.34 with a median of 1.07.
- In group II, the Kt/v ranged from 0.89 to 1.41 with a median of 1.20.
- Kt/v was statistical significant between 2 groups being higher in group II as compared to group

Kt/v: Table (VI)

- In group Ia, the Kt/v ranged from 0.87 – 1.30 with a mean of 1.11 ± 0.13.
- In group Ib, the Kt/v ranged from 0.89 – 1.34 with a mean of 1.07 ± 0.13.
- In group IIa, the Kt/v ranged from 0.97 – 1.41 with a mean of 1.22 ± 0.14.
- In group IIb, the Kt/v ranged from 0.89 – 1.32 with a mean of 1.11 ± .13.
- Kt/v was statistically significant between 4 groups being higher in group (IIa) as compared to the other 3 groups.

Table (I): Comparison between Group I & Group II & Group III according to TGF beta

	Diabetic (n = 30)	Non diabetic (n = 30)	Control (n = 15)	$^{KW}\chi^2$	p
TGF beta					
Min. – Max	7985.50 - 39222.50	6676.3 - 30088.7	4790.4 - 23089.6	30.509*	<0.001*
Mean ± SD	23450.6 ± 8265.5	15785.3 ± 7200.6	8813.2 ± 5757.3		
Median	24504.90	14073.35	6887.40		
Sig .bet .Grps	$p_1 = 0.001^*$, $p_2 < 0.001^*$, $p_3 < 0.001^*$				

$^{KW}\chi^2$: Chi square for Kruskal Wallis test

Sig. bet. grps was done using Mann Whitney test

p_1: p value for comparing between diabetic and non diabetic

p_2: p value for comparing between diabetic and control

p_3: p value for comparing between non diabetic and control

***:** Statistically significant at $p \leq 0.05$

(Table II): Comparison between the four Subgroups (Ia, Ib, IIa, IIb) & Control group III according to TGF beta

- In group Ia, the serum TGF beta ranged from 7985.5 pg/ml to 33084.3 pg/ml with a mean of 19624.0 ± 6394.1 pg /ml.
- In group Ib, the serum TGF beta ranged from 8139.5 pg/ml to 39222.5 pg/ml with a mean of 27277.2 ± 8321.9 pg/ml.
- In group IIa the serum TGF beta ranged from 6676.3 pg/ml to 30088.7 pg/ml with a mean of 12327.9 ± 7355.9pg/ml.
- Group IIb the serum TGF beta ranged from 8795.3 pg/ml to 29442.4 pg/ml with a mean of 19242.6 ± 5261.2 pg/ml.
- In group III (Healthy Control), the serum TGF beta ranged from 4790.4 pg/ml to 230896 pg/ml with a mean of 8813.2 ± 5757.3pg/ml.
- Serum TGF beta was statistical significant between 5 groups being higher in group Ib as compared to group Ia as compared to group IIb as compared to group IIa as compared to group III (Healthy control)

(Table II): Comparison between Group I & Group II according to U/S Doppler AVF

U/S Doppler A.V fistula	Diabetic (n = 30)		Non diabetic (n = 30)		Test of sig.	p
	No.	%	No.	%		
Central venous system stenosis	9	30.0	6	20.0	$\chi^2 = 3.455$	0.120
Thrombosis	1	3.3	5	16.7	$\chi^2 = 2.963$	^{FE}p =0.195
Aneurysm	3	10.0	4	13.3	$\chi^2 = 0.162$	^{FE}p = 1.000
Volume						
Min. – Max	350.0 – 1400.0		500.0 – 1400.0			
Mean ± SD	708.33 ± 272.63		825.0 – 276.91		Z = 1.739	0.082
Median	600.0		775.0			
Vein diameter						
Min. – Max	0.40 - 0.80		0.40 – 0.90			
Mean ± SD	0.61 ± 0.11		0.69 ± 0.11		t =2.695*	0.009*
Median	0.60		0.70			
Osteum						
Min. – Max	0.50 – 0.90		0.50 – 1.0			
Mean ± SD	0.77 ± 0.11		0.72 ± 0.14		t = 1.330	0.189
Median	0.80		0.70			

χ^2: Chi square test

MC: Monte Carlo test

Z: Z for Mann Whitney test

t: Student t-test

*: Statistically significant at p ≤ 0.05

(Table III): Comparison between the four Subgroups (Ia, Ib, IIa, IIb) according to U/S Doppler A.V fistula

	Diabetic (Group I)				Non Diabetic (Group II)				Test of sig.	P
	A.V.F 3 – 6 months (n = 15) (Group Ia)		A.V.F >1 year (n = 15) (Group Ib)		A.V.F 3 – 6 months (n = 15) (Group IIa)		A.V.F >1 year (n = 15) (Group IIb)			
	No.	%	No.	%	No.	%	No.	%		
Central venous system stenosis	4	26.7	5	33.3	4	26.7	2	13.3	$\chi^2 = 6.128$	0.116
Thrombosis	0	0	1	6.7	1	6.7	4	26.7	$\chi^2 = 6.667$	0.142
Aneurysm	1	6.7	2	13.3	0	0.0	4	26.7	$\chi^2 = 4.948$	0.185
Volume										
Min. – Max	350.0 – 1300.0		400.0 – 1400.0		500.0 – 1300.0		500.0 – 1400.0			
Mean ± SD	753.33 ± 286.90		663.33 ± 259.44		823.33 ± 235.94		826.67 ± 321.20		$^{KW}\chi^2 = 4.281$	0.233
Median	700.0		600.0		800.0		700.0			
Vein diameter										
Min. – Max	0.40 – 0.80		0.40 – 0.80		0.40 – 0.90		0.50 - .080			
Mean ± SD	0.63 ± 0.11		0.59 ± 0.11		0.70 ± 0.14		0.69 ± 0.08		F= 2.708	0.054
Median	0.60		0.60		0.70		0.70			
Osteum										
Min. – Max	0.50 – 0.90		0.60 – 0.90		0.50 – 0.90		0.50 – 1.0			
Mean ± SD	0.77 ± 0.12		0.76 ± 0.11		0.70 ± 0.12		0.74 ± 0.15		F = 0.883	0.456
Median	0.80		0.80		0.70		0.80			

χ^2: Chi square test

F: F test (ANOVA)

$^{KW}\chi^2$: Chi square for Kruskal Wallis test

(Table IV): Comparison between Group I & Group II according to Kt/v

	Diabetic (n = 30)	Non diabetic (n = 30)	t	P
Kt/v				
Min. – Max	0.87 – 1.34	0.89 – 1.41		
Mean ± SD	1.09 ± 0.13	1.17 ± 0.14	2.110*	0.039*
Median	1.07	1.20		

t: Student t-test

*: Statistically significant at $p \leq 0.05$

(Table V): Comparison between the four Subgroups (Ia, Ib, IIa, IIb)according to Kt/v

	Diabetic (Group I)		**Non Diabetic (Group II)**		**F**	**P**
	A.V.F 3 – 6 months (n = 15) (Group Ia)	**A.V.F >1 year (n = 15) (Group Ib)**	**A.V.F 3 – 6 months (n = 15) (Group IIa)**	**A.V.F >1 year (n = 15) (Group IIb)**		
Kt/v						
Min. – Max	0.87 – 1.30	0.89 – 1.34	0.97 – 1.41	0.89 – 1.32		
Mean ± SD	1.11 ± 0.13	1.07 ± 0.13	1.22 ± 0.14	1.11 ± .13	3.594	0.019*
Median	1.12	1.05	1.24	1.10		
p_1	0.815		0.113			
p_2			0.123	0.836		

F: F test (ANOVA)

Sig. bet. grps was done using Post Hoc test (Tukey) test

p_1: p value for comparing between A.V.F 3 – 6 months and >1year in each diabetic and non-diabetic group

p_2: p value for comparing between diabetic and non-diabetic in each A.V.F 3 – 6 months and >1 year

* : Statistically significant at p ≤ 0.05

Table (VI): Correlation between TGF Beta with different studied parameters for each group and total patients

	TGF Beta					
	Diabetic (Group I)		**Non Diabetic (Group II)**		**Total patients**	
	r_s	**P**	**r_s**	**P**	**r_s**	**p**
Duration of dialysis (months)	0.456*	0.009	0.420*	0.015*	0.556*	<0.001*
U/S doppler AVF						
Volume	-0.230	0.113	-0.309	0.097	-0.295	0.216
Vein diameter	-0.479*	0.007	-0.202	0.283	-0.481*	<0.001
Osteum	-0.217	0.250	-0.288	0.225	-0.129	0.325
Kt V	-0.385*	0.035	-0.453*	0.012	-0.509*	<0.001*
FBS	0.362*	0.050	0.155	0.415	0.531*	<0.001
Cholesterol	0.501*	0.005	0.017	0.930	0.570*	<0.001

r_s: Spearman coefficient *: Statistically significant at p ≤ 0.05

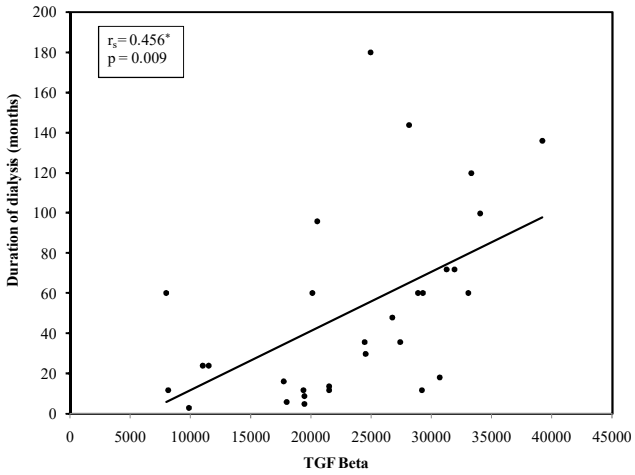

Figure (1): Correlation between TGF Beta with Duration of dialysis (months) for Diabetic group (Group I)

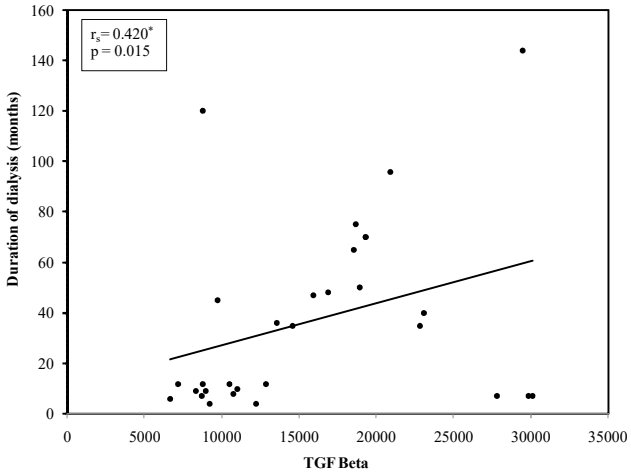

Figure (2): Correlation between TGF Beta with Duration of dialysis (months) for Non Diabetic group (Group II)

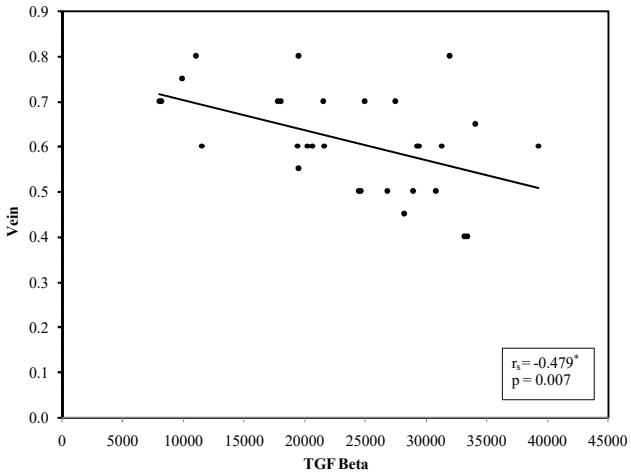

Figure (3): Correlation between TGF Beta with Vein diameter for Diabetic group (Group I)

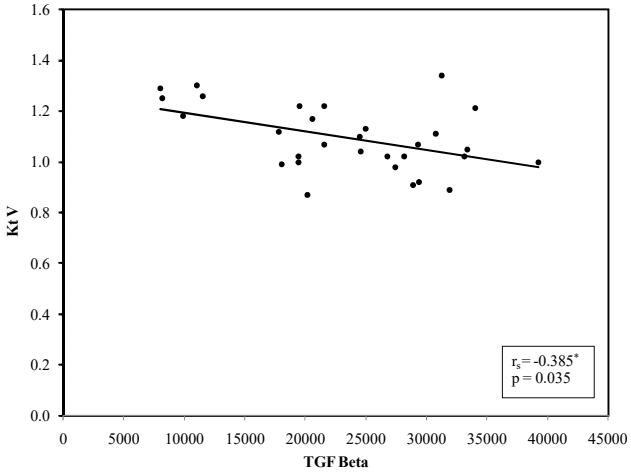

Figure (4): Correlation between TGF Beta with Kt/V for Diabetic group (Group I)

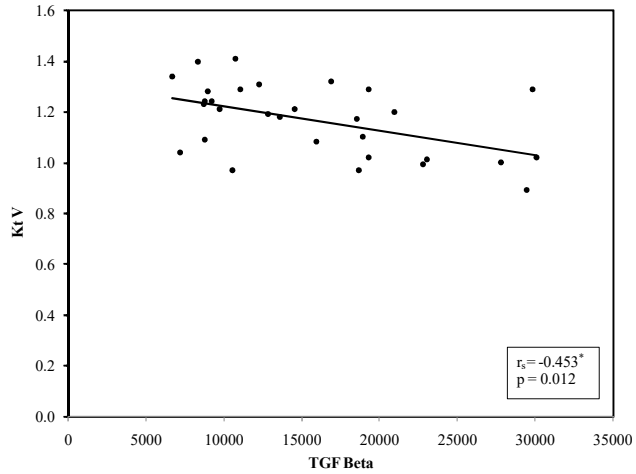

Figure (5): Correlation between TGF Beta with Kt/V for Non Diabetic group (Group II)

Discussion:

The main etiology of ESRD observed in our study was diabetic kidney disease (43%) or hypertensive nephropathy (35%) which is supported by what observed by Robert N et al [18] who found that (43.8%) of their patients had ESRD secondary to diabetic nephropathy and (26.8%) due to hypertensive nephropathy.

It was observed in this study that there was statistical significant higher incidence of history of arteriovenous fistula failure in diabetic patients in comparing with non-diabetic patients .Other studies supported our finding like Renan Nunes da Cruz et al [19] and they found that diabetic patients had shorter mean duration of AVF patency and lower rate of access survival.

Huijbregts HJTet al[20] found that hemodialysis patients with diabetes can be expected to have reduced primary functional native AVF patency rates with high failure rate.

An increase in the level of serum (TGF-β) in the diabetic group was observed in the present study in comparison to non-diabetic group and to healthy controls. In agreement with our results Ahmed KYet al [21] found higher circulating serum levels of TGF beta 1 in patients with diabetes type 1 and type 2 and in those with diabetic nephropathy than the non-diabetics

Ibrahim.S et al [22]strongly support the hypothesis that hyperglycemia may trigger the activation of TGF beta 1 which lead to increase the serum level of TGF beta 1 in type 2 diabetic patients than healthy control.

According to AVF vein diameter in this study it was observed that the vein diameter (arterialized) was statistical significant decreased in diabetic ESRD group in compared to the non-diabetic ESRD group, In agreement with our results Conte MS et al[23] found that diabetes was a significant, negative predictor of venous remodeling over the 24-week study (P = .02). The model-predicted change in lumen diameter from 2 to 24 weeks was -0.7 mm in diabetic patients (n = 11) and +2.4 mm in non-diabetic patients (n = 15), a difference of 3.1 mm.

A significant decrease in the Kt/v in diabetic ESRD group in compared to non-diabetic ESRD group depending on high incidence of arteriovenous fistula stenosis in diabetic group, in agreement with our findings Robbin ML et al [24] revealed that patients with diabetes were significantly less likely to have a well-functioning AVF than patients without diabetes which is important for adequate hemodialysis.

An increase in the level of serum (TGF-β) in the diabetic patients with AFV more than 1 year (Group Ib) was observed in the present study in comparison to other groups. Weiss MF et al [25] who found that oxidative hyperactivity in the uremic status usually leads

to an increased amount of circulating and tissue inflammatory molecules, Interaction with dialysis membranes have also been reported as an important cause leading to oxidative stress, resulting in an increased expression of endothelin-1, which has been associated with intimal hyperplasia and smooth muscle cell vasoconstriction. TGF-β and PDGF have also been implicated in intimal hyperplasia but seem to be clustered at the venous end of the failed AV access.

It was observed in our study that hemodialysis adequacy (kt/v) of the non-diabetic group with AVF duration 3-6 months (Group IIa) was statistically significantly higher in comparing with the other 3 groups. This is supported by Anees M, et al[26] who found that non-diabetic patients had a better quality of life (QOL) as compared to diabetic patients plus that duration of dialysis had a reverse correlation with the overall QOL.

It was observed in the present study that the level of TGF beta is significantly positively correlated with duration of dialysis in both diabetic & non-diabetic group, consistent with our findingsLin CC et al[27] strongly support that Interaction with dialysis membranes have also been reported as an important cause leading to oxidative stress leads to an increased amount of circulating and tissue inflammatory molecules such as TGF beta & PDGF have also been implicated in intimal hyperplasia of the AVF

It was observed in the present study that the level of TGF beta is significantly negatively correlated with vein diameter of AVF in diabetic group. Consistent with our findings Stracke S et al [28] found that there is a venous neointimal thickening of the arteriovenous fistulas represents a local inflammatory process and appears to be associated with increased expression of TGF-beta1 and IGF-I., TGF-beta1 may be an important trigger of ECM production and deposition.

It was observed in the present study that the level of TGF-beta is significantly negatively correlated with Kt/v in both diabetic & non-diabetic group.

It was observed in the present study that the level of TGF-beta is significantly positively correlated with fasting blood glucose in diabetic group. Consistent with our findings Garud MS et al[29] demonstrated that in diabetic nephropathy hyperglycemia cause increase in the expression of TGF- β1 genes, TGF- β 1 proteins and their receptors. Increased blood glucose level also activates the TGF- β via activation of glucose transporters (GLUT) which lead to increase level of serum TGF beta 1.

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