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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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Authors are required to disclose any potential conflict of interest. Acknowledgments should list brief statements of assistance, financial support, and prior publication of the study in abstract form, if applicable.

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Contents

Letter From The Editor

Dear Colleague,

In this issue, we have included an interesting Editorial Article about The Use of Insulin Analogues in Clinical Practice in Type 2 Diabetes Mellitus: Results from the Egyptian Sub-Group of the A1chieve Observational Study.

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

Cover of the Journal: The gods and goddesses of the ancient Egyptian civilization including: Isis (goddess of prosperity and female fertility), Min (god of male fertility), ect… Designed by: S.H. Assaad Khalil

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Editorial Article

The Use of Insulin Analogues in Clinical Practice in Type 2 Diabetes Mellitus: Results from the Egyptian Sub-Group of the A1chieve Observational Study.

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Aim:

A_1chieve[1] was a prospective, multinational, non-interventional study conducted with the aim to bridge the gap of available information regarding the safety and efficacy of insulin analogues in routine clinical practice in developing and newly developed countries. Our focus in this abstract is to highlight the safety and efficacy of insulin analogues in an Egyptian cohort of the A_1chieve[1] study.

Methods:

A total of 412 Egyptian patients with type 2 diabetes mellitus (T2DM) initiating biphasic insulin aspart 30, insulin detemir, and insulin aspart alone or in combination, following prior therapy with oral antidiabetics or other insulins, were included in this 6-month observational study.

The primary outcome was the evaluation of serious adverse drug reactions including major hypoglycaemic events. The secondary outcomes were changes in hypoglycaemic events and the following efficacy parameters: HbA_{1c}, fasting plasma glucose (FPG), postprandial plasma glucose (PPPG), systolic blood pressure (SBP), body weight and lipid profile. Quality of life (QoL) was determined using the EQ-5D* questionnaire that evaluates patient concerns with regard to mobility, self-care, usual activity, pain/discomfort and anxiety.

Results:

This analysis included 172 insulin-naïve patients and 240 prior insulin usershaving mean age ± SD 52.8 ± 9.7 years, mean BMI 30.2 ± 4.9 kg/m^2 and mean diabetes duration 10.5 ± 6.8 years. Mean baseline data were reported as follows: HbA_{1c} 9.2 ± 1.8%, FPG 205.7 ± 78.8 mg/dL, post-breakfast PPPG 282.2 ± 92.7 mg/dL and SBP 134.6 ± 16.4 mmHg. Parameters derived from the baseline data in Egypt were comparable to North Africa, Middle East + Gulf and the global A_1chieve data with the exception of mean body weight that was higher in Egypt (87.6 ± 14.1 kg) and Middle East + Gulf (84.4 ± 15.4 kg) compared to the global A_1chieve data (73.3 ± 14.8 kg).

Keywords: Egypt, type 2 diabetes, insulin analogues, A_1chieve study, observational study.

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Physicians reported that 91.5% of Egyptian patients initiated insulin analogues to improve glycemic control. The mean pre-study insulin dose in prior insulin users was 56.4 ± 28.6 U/day and the starting dose was 62.4 ± 28.0 U/day titrated up to 65.8 ± 29.4 U/day. In insulin-naïve patients the mean insulin analogue dose was titrated up to 35.9 ± 23.9 U/day at Week 24. The highest insulin doses at pre-study (66.7 ± 32.3 U/day), baseline (79.6 ± 28.5 U/day) and Week 24 (82.0 ± 29.0 U/day) were reported in prior insulin users receiving insulin aspart + basal insulin.

Overall hypoglycaemia reduced significantly in the entire cohort from baseline to Week 24 (7.67 vs. 1.97 events/patient-year) with no major hypoglycaemic events at Week 24. At Week 12, 1 event of major nocturnal hypoglycaemia in the insulin aspart + basal insulin group was reported. The proportion of prior insulin users reporting overall hypoglycaemia significantly decreased with all treatments. The highest baseline incidence of overall hypoglycaemia (14.41 events/patient-year) and greatest reduction at Week 24 (1.86 events/patient-year) was reported in prior insulin users receiving insulin aspart + basal insulin (change in proportion of patients affected, $p < 0.0001$). Nocturnal hypoglycaemia decreased in prior insulin users receiving biphasic insulin aspart 30 (3.40 to 0.34 events/patient-year; change in proportion of patients affected, $p = 0.01$) and insulin detemir (3.58 to 0 events/patient-year; change in proportion of patients affected, $p = 0.01$). An increase in overall hypoglycaemia was reported in insulin-naïve patients on biphasic insulin aspart 30 (0.28 events/patient-year at baseline vs 1.81 events/patient-year at Week 24) and insulin aspart + basal insulin (2.36 events/patient-year at baseline vs. 7.22 events/patient-year at Week 24); however, the increase in the proportion of patients affected was not significant ($p > 0.3$). This increase was primarily attributed to the occurrence of minor hypoglycaemic events. Nocturnal hypoglycaemia in insulin-naïve patients decreased from 0.87 to 0 events/patient-year in the insulin detemir group (change in proportion of patients affected, $p = 0.12$), while an increase from 0 to 2.89 events/patient-year was reported with insulin aspart + basal insulin (change in proportion of patients affected, $p = 0.19$). The highest baseline hypoglycaemia and consequently the greatest reduction at Week 24 were reported in Egypt and North Africa compared to Middle East + Gulf and global A1chieve data.

In the entire Egyptian cohort, significant reductions in HbA_{1c} (-1.7 ± 1.8%), FPG (-73.9 ± 82.9 mg/dL), post-breakfast PPPG (-110.7 ± 103.7 mg/dL) and SBP (-5.1 ± 16.4 mmHg) were observed ($p < 0.001$). These results were consistent with those reported in North Africa and Middle East + Gulf as well as the global A1chievedata.The change from baseline in HbA_{1c} (-2.4 ± 1.5%), FPG (-99.0 ± 78.0 mg/dL), PPPG (-139.1 ± 96.1 mg/dL) and SBP(-8.6 ± 19.6 mmHg) was more profound in insulin-naïve patients on biphasic insulin aspart 30 compared to other groups. Lipid parameters improved significantly in the entire Egyptian cohort while body weight changed minimally. These findings were in line with data from North Africa, Middle East + Gulf and the global A1chieve data.

QoL improved significantly in Egyptian patients (change in visual analogue scores* 15.1 ± 16.1 points, $p < 0.001$). Pain/discomfort was the QoL parameter that recorded the greatest changein patients experiencing no problems from baseline (40.4%) to Week 24 (64.2 %, $p < 0.0001$)followed by usual activity (59.2% at baseline vs. 76.5% at Week 24, $p < 0.0001$) and anxiety (57.1% at baseline vs. 74.3% at Week 24, $p < 0.0001$).

*The current QoL was measured on a standard 20 cm visual analogue scale. The EQ-5D VAS scores range from 0 to 100 (worst imaginable to best imaginable health). Each health-state dimension can be converted to a single utility value using an EQ-5D value set, the UK VAS set in the A1chieve study, and is anchored by '1.00' representing full health and '0.00' representing the state 'dead'.

Table I. Baseline and 24-week data for effectiveness outcomes in the entire cohort and by pre-study insulin type- Egyptian data

Parameter		Entire cohort (n = 412)	Insulin-naïve (n = 172)	Prior insulin users (n = 240)
HbA_{1c}, %	Baseline	9.2 (1.8)	9.2 (1.9)	9.2 (1.8)
	Week 24	7.6 (1.5)	7.2 (1.0)	7.8 (1.7)
	Change	-1.7 (1.8)	-2.0 (1.6)	-1.4 (1.9)
	p	<0.001	<0.001	<0.001
FPG, mg/dL	Baseline	205.7 (78.8)	200.3 (72.6)	209.4 (82.9)
	Week 24	131.8 (49.3)	118.5 (32.2)	141.2 (56.7)
	Change	-73.9 (82.9)	-81.9 (73.8)	-68.2 (88.4)
	p	<0.001	<0.001	<0.001
PPPG, mg/dL post-breakfast	Baseline	282.2 (92.7)	283.4 (85.0)	281.4 (98.1)
	Week 24	171.6 (54.8)	164.2 (53.1)	176.9 (55.6)
	Change	-110.7 (103.7)	-119.2 (95.5)	-104.5 (109.1)
	p	<0.001	<0.001	<0.001
Body weight, kg	Baseline	87.6 (14.1)	87.5 (12.7)	87.6 (15.1)
	Week 24	87.5 (13.5)	87.3 (12.0)	87.6 (14.5)
	Change	-0.1 (3.2)	-0.2 (3.1)	-0.1 (3.3)
	p	0.537	<0.001	<0.812
SBP, mmHg	Baseline	134.6 (16.4)	134.2 (16.6)	134.9 (16.3)
	Week 24	129.5 (13.7)	128.0 (10.8)	130.5 (15.3)
	Change	-5.1 (16.4)	-6.2 (15.4)	-4.4 (17.1)
	p	<0.001	<0.001	<0.001
Total cholesterol, mg/dL	Baseline	203.5 (44.2)	204.3 (39.7)	202.9 (47.5)
	Week 24	183.5 (36.6)	181.9 (35.4)	184.7 (37.6)
	Change	-20.1 (51.0)	-22.4 (45.7)	-18.3 (54.9)
	p	<0.001	<0.001	<0.001
Triglycerides, mg/dL	Baseline	185.2 (92.4)	192.6 (96.2)	179.6 (89.4)
	Week 24	155.5 (59.0)	155.7 (61.0)	155.4 (57.6)
	Change	-29.7 (77.0)	-36.9 (77.2)	-24.2 (76.6)
	p	<0.001	<0.001	<0.001
HDL cholesterol, mg/dL	Baseline	43.0 (11.1)	43.7 (11.9)	42.4 (10.5)
	Week 24	45.3 (12.5)	44.8 (5.9)	45.8 (15.8)
	Change	2.4 (14.3)	1.0 (11.3)	3.4 (16.2)
	p	0.03	0.421	0.041
LDL cholesterol, mg/dL	Baseline	122.6 (35.5)	123.8 (36.5)	121.8 (34.9)
	Week 24	112.7 (29.6)	114.1 (28.6)	111.6 (30.4)
	Change	-10.0 (36.4)	-9.6 (37.6)	-10.2(35.7)
	p	<0.001	0.028	0.006

Data reported as mean (SD)

Table II. Rates of hypoglycaemia in the entire cohort and by pre-study insulin therapy type-Egyptian data

Parameter		Entire cohort	Insulin-naïve	Prior insulin users
Overall	Baseline	7.67/23.8	1.74/7.0	11.92/35.8
	Week 12	1.61/7.8	1.39/5.7	1.77/9.2
	Week 24	1.97/8.2	1.43/5.8	2.35/10.0
	P	<0.0001	0.822	<0.001
Minor	Baseline	7.16/22.6	1.59/7.0	11.16/33.8
	Week 12	1.58/7.5	1.39/5.7	1.71/8.8
	Week 24	1.97/8.2	1.43/5.8	2.35/10.0
	P	<0.0001	0.822	<0.001
Nocturnal	Baseline	1.89/9.2	0.60/2.9	2.82/13.8
	Week 12	0.30/2.1	0.25/1.3	0.34/2.6
	Week 24	0.28/2.1	0.17/1.3	0.35/2.7
	P	<0.0001	0.45	<0.001
Major	Baseline	0.50/2.2	0.15/0.6	0.76/3.3
	Week 12	0.03/0.3[a]	0/0	0.06/0.4[a]
	Week 24	0/0	0/0	0/0
	p	0.004	1	0.0077

Data reported as event per patient-year/percent with event
p-value is for difference in percent of patients with at least one event at Week 24
[a]1 event of major nocturnal hypoglycaemia reported in 1 patient

Table III - Baseline and 24-week data for effectiveness outcomes by insulin analogue regimen started- Egyptian Data

		Insulin-naïve				Prior insulin users			
		Biphasic insulin aspart 30	**Insulin detemir**	**Insulin aspart alone**	**Insulin aspart +basal**	**Biphasic insulin aspart 30**	**Insulin detemir**	**Insulin aspart alone**	**Insulin aspart +basal**
Insulin dose, U/day	n	47	105	1	11	84	40	0	46
	Prestudy	-	-	-	-	53.5 (26.2)	33.3 (22.7)	-	66.7 (32.3)
	Baseline	44.7 (13.8)	17.8 (9.2)	15.0 (0)	69.3 (20.1)	60.6 (23.1)	25.5 (11.6)	-	79.6 (28.5)
	Week 24	48.5 (15.9)	23.3 (14.7)	35.0 (0)	70.8 (27.7)	67.6 (26.8)	27.0 (11.9)	-	82.0 (29.0)
HbA_{1c}, %	n	37	82	1	9	58	35	0	38
	Baseline	9.8 (1.8)	8.8 (1.7)	8.3 (0)	9.1 (2.8)	9.4 (1.5)	8.7 (1.9)	-	9.4 (1.8)
	Week 24	7.4 (0.9)	7.0 (1.0)	8.5 (0)	6.8 (0.7)	7.8 (1.3)	7.9 (2.0)	-	7.5 (1.2)
	Change	-2.4 (1.5)	-1.8 (1.6)	0.2 (0)	-2.3 (2.4)	-1.6 (1.6)	-0.8 (2.1)	-	-1.9 (1.7)
	p	<0.001	<0.001	<0.001	0.021	<0.001	0.029		<0.001
FPG, mg/dL	n	43	95	1	8	76	39	0	41
	Baseline	221.3 (66.5)	185.1 (66.7)	171.0 (.)	231.8 (99.8)	207.7 (73.8)	181.8 (80.8)	-	211.3 (92.7)
	Week 24	122.3 (39.6)	114.6 (27.0)	191.0 (.)	120.5 (28.2)	133.1 (42.2)	132.9 (45.6)	-	137.9 (46.7)
	Change	-99.0 (78.0)	-70.5 (65.5)	20.0 (.)	-111.3 (111.6)	-74.6 (69.7)	-48.9 (87.7)	-	-73.4 (96.3)
	p	<0.001	<0.001	<0.001	0.0026	<0.001	<0.001		<0.001
PPPG, mg/dL	n	43	94	1	8	75	37	0	39
	Baseline	303.3 (73.3)	270.4 (88.8)	372.0 (.)	311.0 (101.1)	282.4 (86.7)	255.4 (93.9)	-	280.6 (112.1)
	Week 24	164.2 (59.0)	164.0 (50.3)	299.0 (.)	146.6 (32.0)	173.5 (45.6)	182.3 (54.5)	-	167.4 (45.7)
	Change,	-139.1 (96.1)	-106.5 (92.5)	-73.0 (.)	-164.4 (113.6)	-108.9 (88.7)	-73.1 (105.4)	-	-113.2 (114.8)
	p	<0.001	<0.001	<0.001	0.005	<0.001	<0.001		<0.001
Body weight, kg	n	40	93	1	9	73	39	0	41
	Baseline	89.0 (14.6)	87.2 (11.5)	61.0 (-)	88.1 (13.7)	89.2 (14.4)	89.2 (16.5)	-	88.6 (15.5)
	Week 24	88.6 (13.8)	87.0 (11.0)	64.0 (-)	88.9 (13.2)	89.6 (13.8)	88.9 (15.6)	-	88.2 (15.0)
	Change	-0.4 (3.4)	-0.2 (3.1)	3.0 (0)	0.7 (3.0)	0.4 (4.3)	-0.3 (2.4)	-	-0.5 (2.6)
	p	0.45	0.594	<0.001	0.479	0.434	0.408		0.249
SBP, mmHg	n	43	94	1	9	77	37	0	42
	Baseline	137.3 (20.2)	133.4 (15.0)	140.0 (-)	135.0 (18.4)	138.3 (14.4)	128.8 (11.4)	-	130.7 (19.0)
	Week 24	128.7 (10.9)	127.8 (11.0)	140.0 (-)	130.6 (10.7)	131.3 (18.4)	130.1 (14.1)	-	128.2 (11.2)
	Change	-8.6 (19.6)	-5.6 (13.2)	0.0 (-)	-4.4 (18.3),	-7.0 (19.1)	1.4 (12.5)	-	-2.5 (13.3)
	p	0.006	<0.001	<0.001	0.486	0.002	0.515		0.229
Total cholesterol, mg/dL	n	16	65	1	5	42	28	0	23
	Baseline	229.8 (26.6)	198.0 (40.2)	182.0 (.)	208.2 (52.5)	209.8 (50.6)	202.9 (40.9)	-	182.2 (38.5)
	Week 24	171.9 (59.0)	181.8 (23.3)	320.0 (.)	185.2 (25.6)	184.1 (42.2)	185.6 (33.7)	-	179.2 (28.0)
	Change	-57.9 (62.2)	-16.2 (32.1)	138.0 (.)	-23.0 (52.0)	-25.7 (54.7)	-17.3 (43.1)	-	-3.0 (37.2)
	p	0.002	<0.001	<0.001	0.379	0.004	0.043		0.702
Triglycerides, mg/dL	n	16	65	1	5	42	29	0	23
	Baseline	238.3 (102.6)	183.2 (94.4)	148.0 (.)	159.6 (93.8)	186.6 (74.6)	179.6 (87.6)	-	148.1 (101.9)
	Week 24	149.1 (56.6)	157.8 (63.5)	171.0 (.)	125.4 (50.9)	160.1 (54.1)	158.2 (63.2)	-	133.1 (56.4)
	Change	-89.2 (101.2)	-25.4 (69.3)	23.0 (.)	-34.2 (43.9)	-26.5 (76.2)	-21.4 (68.8)	-	-15.0 (65.3)
	p	0.003	0.004	<0.001	0.156	0.03	0.105		0.282
HDL cholesterol, mg/dL	n	9	60	1	5	32	26	0	21
	Baseline	47.6 (20.3)	43.3 (10.8)	35.0 (.)	45.2 (6.2)	40.6 (7.7)	44.1 (13.0)	-	43.7 (9.6)
	Week 24	43.3 (5.7)	45.1 (5.6)	38.0 (.)	46.0 (10.1)	46.0 (17.2)	46.5 (21.0)	-	47.1 (12.4)
	Change	-4.2 (19.0)	1.8 (10.4)	3.0 (.)	0.8 (6.8)	5.3 (16.2)	2.4 (23.4)		3.4 (9.6)
	p	0.523	0.199	<0.001	0.804	0.073	0.601		0.118
LDL cholesterol, mg/dL	n	9	59	1	5	32	26	0	20
	Baseline	155.3 (17.8)	117.8 (36.2)	117.0 (.)	140.8 (46.7)	127.8 (44.3)	119.2 (28.9)	-	108.3 (25.7)
	Week 24	126.0 (25.1)	109.8 (24.1)	248.0 (.)	119.8 (20.8)	109.6 (31.7)	111.4 (28.8)	-	105.1 (29.7)
	Change	-29.3 (34.4)	-8.0 (32.0)	131.0 (.)	-21.0 (56.8)	-18.1 (34.1)	-7.8 (31.1)	-	-3.2 (34.7)
	p	0.034	0.061	<0.001	0.455	0.005	0.214		0.683

Data reported as mean (SD)

Table IV. Rates of hypoglycaemia in the entire cohort and by pre-study insulin regimen type-Egyptian data

		Insulin-naive				Prior insulin users			
		Biphasic insulin aspart 30	**Insulin detemir**	**Insulin aspart alone**	**Insulin aspart +basal**	**Biphasic insulin aspart 30**	**Insulin detemir**	**Insulin aspart alone**	**Insulin aspart +basal**
Overall	Baseline	0.28/2.1	2.10/8.6	0/0	2.36/9.1	10.21/29.8	11.38/30.0	0/0	14.41/45.7
	Week 12	0.89/4.5	1.35/4.2	0/0	5.20/30.0	1.63/8.8	0.65/5.0	0/0	2.42/11.6
	Week 24	1.81/7.0	0.55/3.2	0/0	7.22/22.2	2.70/13.0	0.67/2.6	0/0	1.86/ 4.8
	p[a]	0.3451	0.1399	-	0.5658	0.0126	0.0015	-	<0.0001
Minor	Baseline	0.28/ 2.1	1.86/8.6	0/0	2.36/9.1	9.29/28.6	10.73/25.0	0/0	12.72/41.3
	Week 12	0.89/4.5	1.35/4.2	0/0	5.20/30.0	1.63/8.8	0.65/5.0	0/0	2.12/9.3
	Week 24	1.81/7.0	0.55/3.2	0/0	7.22/22.2	2.70/13.0	0.67/2.6	0/0	1.86/4.8
	p[a]	0.3451	0.1399	-	0.5658	0.02	0.0069	-	<0.0001
Nocturnal	Baseline	0/0	0.87/3.8	0/0	0/0	3.40/14.3	3.58/17.5	0/0	2.83/13.0
	Week 12	0/0	0.27/1.0	0/0	1.30/10.0	0.16/1.3	0.65/5.0	0/0	0.60/4.7[a]
	Week 24	0/0	0/0	0/0	2.89/22.2	0.34/2.6	0/0	0/0	0.31/2.4
	p[a]	-	0.1231	-	0.1895	0.0104	0.0117	-	0.1127
Major	Baseline	0/0	0.25/1.0	0/0	0/0	0.93/2.4	0.65/ 5.0	0/0	1.70/8.7
	Week 12	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.30/2.3[a]
	Week 24	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
	p[a]	-	1	-	-	0.4978	0.4937	-	0.118

Data reported as event per patient-year/percent with event p-value is for difference in percent of people with at least one event
[a]1 event of major nocturnal hypoglycaemia reported in 1 patient at Week 12

Table V. Baseline and 24-week data for effectiveness and safety outcomes by entire cohort

Parameter		**Egypt (n = 412)**	**North Africa (n = 4033)**	**Middle East + Gulf (n = 14,896)**	**Global study (n =44,661)**
HbA1c, %	Baseline	9.2 (1.8)	9.5 (1.8)	9.6 (1.7)	9.5 (1.7)
	Week 24	7.6 (1.5)	7.9 (1.4)	7.4 (1.1)	7.4 (1.1)
	Change	-1.7 (1.8)	-1.6 (1.9)	-2.2 (1.6)	-2.1 (1.7)
	p	<0.001	<0.001	<0.001	<0.001
FPG, mg/dL	Baseline	205.7 (78.8)	205.1 (75.4)	204.1 (66.3)	197.2 (63.6)
	Week 24	131.8 (49.3)	142.5 (50.0)	126.7 (33.3)	128.6 (35.0)
	Change	-73.9 (82.9)	-62.7 (85.4)	-77.4 (64.9)	-68.6 (63.1)
	p	<0.001	<0.001	<0.001	<0.001
PPPG, mg/dL post-breakfast	Baseline	282.2 (92.7)	265.9 (84.3)	277.5 (82.1)	272.5 (79.4)
	Week 24	171.6 (54.8)	187.2 (63.4)	166.4 (43.8)	175.6 (52.2)
	Change	-110.7 (103.7)	-78.7 (99.7)	-111.1 (81.1)	-96.9 (80.6)
	p	<0.001	<0.001	<0.001	<0.001
Body weight, kg	Baseline	87.6 (14.1)	75.4 (13.3)	84.4 (15.4)	73.3 (14.8)
	Week 24	87.5 (13.5)	76.2 (12.8)	84.0 (14.4)	73.3 (14.1)
	Change	-0.1 (3.2)	0.9 (3.9)	-0.4 (4.4)	0.1 (3.7)
	p	0.537	<0.001	<0.001	<0.001
SBP, mmHg	Baseline	134.6 (16.4)	133.1 (18.2)	134.9 (17.3)	134.2 (17.8)
	Week 24	129.5 (13.7)	131.0 (19.2)	128.5 (13.2)	127.9 (13.5)
	Change	-5.1 (16.4)	-2.1 (20.9)	-6.4 (16.5)	-6.3 (17.1)
	p	<0.001	<0.001	<0.001	<0.001
Total cholesterol, md/dL	Baseline	203.5 (44.2)	181.9 (45.9)	205.5 (45.6)	205.4 (49.5)
	Week 24	183.5 (36.6)	174.4 (39.0)	181.8 (32.0)	185.4 (37.6)
	Change	-20.1 (51.0)	-7.6 (46.9)	-23.7 (43.9)	-20.0 (45.3)
	p	<0.001	<0.001	<0.001	<0.001
Triglycerides, mg/dL	Baseline	185.2 (92.4)	146.3 (83.0)	193.3 (89.5)	184.2 (95.1)
	Week 24	155.5 (59.0)	136.1 (59.6)	161.9 (59.5)	155.7 (64.4)
	Change	-29.7 (77.0)	-10.1 (76.5)	-31.4 (81.5)	-28.4 (83.5)
	p	<0.001	<0.001	<0.001	<0.001
HDL cholesterol, mg/dL	Baseline	43.0 (11.1)	43.1 (15.9)	42.2 (12.9)	44.4 (15.5)
	Week 24	45.3 (12.5)	44.3 (15.1)	43.5 (11.2)	46.5 (15.4)
	Change	2.4 (14.3)	1.2 (19.4)	1.4 (13.4)	2.1 (15.5)
	p	0.03	0.046	<0.001	<0.001
LDL cholesterol, mg/dL	Baseline	122.6 (35.5)	111.5 (47.9)	122.1 (38.1)	121.2 (40.6)
	Week 24	112.7 (29.6)	106.1 (42.2)	106.0 (29.5)	106.5 (33.4)
	Change	-10.0 (36.4)	-5.4 (56.6)	-16.1 (38.8)	-14.6 (40.5)
	p	<0.001	0.003	<0.001	<0.001

Data reported as mean (SD)

Table VI. Rates of hypoglycaemia by entire cohort

Parameter		Egypt (n = 412)	North Africa (n = 4033)	Middle East + Gulf (n = 14,896)	Global study (n =44,661)
Overall	Baseline	7.67/23.8	8.14/18.6	3.94/10.1	3.11/8.9
	Week 12	1.61/7.8	4.94/16.2	2.18/8.0	1.85/6.7
	Week 24	1.97/8.2	4.09/13.6	2.1/7.6	1.61/5.9
	P	<0.0001	<0.001	<0.001	<0.0001
Minor	Baseline	7.16/22.6	6.96/18.0	3.36/9.5	2.79/8.5
	Week 12	1.58/7.5	4.85/16.2	2.16/8.0	1.84/6.7
	Week 24	1.97/8.2	4.03/13.4	2.09/7.6	1.60/5.8
	P	<0.0001	<0.001	<0.001	<0.0001
Nocturnal	Baseline	1.89/9.2	3.23/11.4	1.07/4.6	0.93/4.0
	Week 12	0.30/2.1	1.35/5.7	0.56/2.8	0.42/2.0
	Week 24	0.28/2.1	1.26/5.3	0.57/2.9	0.36/1.8
	P	<0.0001	<0.001	<0.001	<0.0001
Major	Baseline	0.50/2.2	1.18/5.3	0.58/2.5	0.33/1.5
	Week 12	0.03/0.3[a]	0.09/0.4	0.02/0.1	0.01/0.06
	Week 24	0/0	0.06/0.2	0.01/0.1	0.01/0.03
	p	0.004	<0.001	<0.001	<0.0001

Data reported as event per patient-year/percent with event

p-value is for difference in percent of patients with at least one event at Week 24

[a]1 event of major nocturnal hypoglycaemia reported in 1 patient

Serious adverse event report

At Week 12, 1 prior insulin user receiving insulin aspart (80 IU) + basal insulin (insulin detemir 30 IU) experienced 1 event of major nocturnal hypoglycaemia.

Conclusions:

Insulin analogue therapy resulted in improved glycaemic control and a significant overall decrease in hypoglycaemia without any major concerns of safety in Egyptian T2DM patients. These results provide evidence that initiating or switching to insulin analogues could be beneficial in long-term T2DM management irrespective of prior insulin use.

Reference:

1. **Home P, Naggar NE, Khamseh M, et al.** An observational non-interventional study of people with diabetes beginning or changed to insulin analogue therapy in non-Western countries: the A1chieve study. Diabetes Res Clin Pract 2011;94(3):352-63.

Original Article

NovoMix®30 in Clinical Practice for Type 2 Diabetes Mellitus Management: Results from the Egyptian Sub-Group Patients of the A1chieve Observational Study

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Abstract:

Aim: To determine the safety, effectiveness and effects on quality of life (QoL) following therapy with biphasic insulin aspart 30 (BIAsp 30) in patients with type 2 diabetes mellitus (T2DM) from Egypt as part of the 24-week, prospective, non-interventional A1chieve study (conducted across four continents). **Methods:** Patients with T2DM who required insulin and whose physicians had decided to prescribe the premixed insulin analog BIAsp 30 were eligible for this sub-analysis. A total of 412 T2DM patients initiating insulin analog therapy after oral glucose-lowering drugs or switching from another insulin were recruited from Egypt, of which 131 patients were prescribed BIAsp 30 either once- or twice-daily. **Results:** In the Egyptian cohort receiving BIAsp 30, baseline glycemic control was poor with a mean (±SD [standard deviation]) glycated hemoglobin A_{1c} (HbA_{1c}) of 9.5 ± 1.6%, fasting plasma glucose (FPG) of 212.6 ± 71.3 mg/dL, and postprandial plasma glucose (PPPG) of 290.0±82.4 mg/dL. Significant reductions occurred in mean (±SD) HbA_{1c} (-1.9 ± 1.6%), FPG (-83.4 ± 73.4 mg/dL), and PPPG (-119.9 ± 92.2 mg/dL) levels after 24 weeks ($p<0.001$). These results were consistent with those reported in North Africa and Middle East + Gulf as well as the global A1chieve data. Blood glucose control improved in both insulin-naïve patients and prior insulin users in the Egyptian cohort. In insulin-naïve patients, the change from baseline in mean (±SD) HbA_{1c}, FPG, and PPPG levels was -2.4 ± 1.5%, -99.0 ± 78.0 mg/dL, and -139.1 ± 96.1 mg/dL, respectively, at Week 24. In prior insulin users, the change from baseline in mean (±SD) HbA_{1c}, FPG, and PPPG levels was -1.6 ±1.6%, -74.6 ± 69.7 mg/dL, and -108.9 ± 88.7 mg/dL, respectively, at Week 24. No major hypoglycemic events were reported in the Egyptian cohort, supporting the results from North Africa, Middle East + Gulf, and the global A1chieve cohort. Overall and minor hypoglycaemia was observed to increase slightly in insulin-naïve patients and reduce in prior insulin users in the Egyptian cohort; no nocturnal hypoglycemic events were reported in insulin-naïve patients and it reduced among prior insulin users. No significant body weight gain was reported for the Egyptian cohort or Middle East + Gulf while a statistically significant body weight gain was reported for North Africa and the global A_1 chieve cohort. Mean lipid profile and systolic blood pressure (SBP) improved significantly from baseline to Week 24 in the Egyptian cohort, in line with data from North Africa, Middle East + Gulf, and the global A1chieve cohort. Mean lipid levels were observed to improve in insulin-naïve patients (except for high-density lipoprotein cholesterol) and prior insulin users in the Egyptian cohort. Significant improvements in QoL were reported after 24 weeks (change in visual analog scores = +15.9 ± 15.8). QoL improved in both insulin-naïve patients and prior insulin users (change in visual analog scores = +16.6 ± 13.0 and +15.5 ± 17.3, respectively). The most significant improvement was noted for pain in the entire cohort and insulin-naïve patients, while anxiety was most improved in prior insulin users. **Conclusions:** BIAsp 30 therapy appears safe and effective in achieving glycemic control and resulted in improved QoL in Egyptian T2DM patients.

Keywords: BIAsp 30, observational study, type 2 diabetes mellitus, Egypt, glycaemic control.

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Introduction:

The worldwide prevalence of diabetes is estimated to increase dramatically from 366 million diabetes patients in 2011 to 552 million by 2030.[1] Diabetes accounted for 4.6 million deaths worldwide in 2011 and 183 million people (50%) with diabetes remain undiagnosed.[2] Currently, type 2 diabetes mellitus (T2DM) constitutes over 90% of all diabetes cases worldwide.[3]

Diabetes has greatest prevalence in developing countries resulting from population growth, increasing rates of obesity and physical inactivity.[4-6] The Middle East and North Africa (MENA) region reported the highest regional prevalence of diabetes for 2011 (after age standardization to the world population).[1] In 2010, the International Diabetes Federation estimated approximately 27 million diabetes patients in the MENA region.[7] Moreover, the high prevalence of diabetes is anticipated to continue in the MENA region with an estimated 94% increase (51.7 million) in the number of people with diabetes from 2010 to 2030, second highest, with the African region leading in the world.[7]

In 2010, Egypt was among the six countries in the MENA region listed among the world's ten highest in prevalence rates for both diabetes and the impaired glucose tolerance that leads to diabetes.[8] Furthermore, in 2011, Egypt reported the largest number of people (7.3 million) with diabetes in the MENA region.[1] The socio-economic changes and westernisation together with aging of populations has resulted in such dramatic increase in the diabetes prevalence.[8] In Egypt, emerging evidence showed that diabetes mellitus, obesity, hypertension, and hyperlipidemia contribute to national morbidity and mortality, representing about 26% of all deaths related to chronic diseases.[9]

Due to the progressive nature of T2DM, most patients with this disease ultimately require insulin therapy[10,11], which was further supported by the United Kingdom Prospective Diabetes Study stating that > 60% of T2DM patients will need insulin within 5 years of diagnosis.[12,13] Intensive therapy resulting in good glycemic control reduces micro- and macrovascular complications associated with T2DM.[12,14] Measurement of glycated hemoglobin A_{1c} (HbA_{1c}) still remains the standard criterion for evaluating glycemic control.[15]

The American Diabetes Association recommends target HbA_{1c} levels < 7.0% in most patients to reduce the incidence of microvascular disease.[16] This target HbA_{1c} level can be achieved with a mean plasma glucose of ~150–160 mg/dL; ideally, fasting and premeal glucose maintained at <130 mg/dL and postprandial glucose at <180 mg/dL.[17] Through careful adherence to diabetes management and a multifactorial treatment plan focusing on modulating fasting plasma glucose (FPG) and postprandial plasma glucose (PPPG), the required target HbA_{1c} levels can be achieved.[15] However, it often remains a challenge to achieve the target HbA_{1c} level (< 7.0%) owing to multiple issues such as insecurity about insulin treatment, fear of hypoglycemia, weight gain, delayed initiation of insulin treatment, and ignoring PPPG increase.[18,19] The inability to achieve glycemic control, when combined with the disorder's increasing prevalence, results in increasing morbidity and mortality among diabetes patients.[15,20]

In the Egyptian insulin market, combined insulin (biphasic human insulin [BHI]) has been the major driver (by volume), according to IMS statistical research studies on purchases in the Egyptian private pharmacy market between January 2010 and December 2011.[21] BHI is associated with relatively high risks of hypoglycaemia, probably owing to limitations in its pharmacokinetic profile.[22] A previous study showed that biphasic insulin analogs provide improved PPPG control with a reduced number of hypoglycemic episodes compared to BHI preparations.[23]

Biphasic insulin aspart 30 (BIAsp 30) is a premix insulin analog formulation containing both soluble and intermediate acting insulin (30% soluble insulin aspart [IAsp] and 70% IAsp protamine crystals) developed to provide diabetic patients with the convenience of administering insulin either immediately before or soon after a meal. BIAsp 30 therapy was shown to improve glycemic control and reduce hypoglycemic rates in the large observational study, IMPROVE.[24,25] Previous randomised controlled trials have demonstrated that BIAsp 30 therapy can result in better glycemic control compared to a basal insulin analog.[26,27]

Maintaining or improving the quality of life (QoL) of patients is also an important aspect of successful diabetes management.[28] BIAsp 30 therapy was reported to positively impact the QoL of T2DM patients in the IMPROVE study.[29]

A_1chieve was the largest observational study conducted to evaluate the safety and effectiveness of initiating or switching to insulin analogs (BIAsp 30, IAsp, and insulin detemir) in a heterogeneous T2DM population across various geographical regions.[30] In this paper, we aimed to evaluate the safety and effectiveness of BIAsp 30 in an Egyptian subgroup of T2DM patients as part of the global A_1chieve study.

Methods:

Study Design:

A_1chieve [30] was a 24-week, international, prospective, multi-center, non-interventional study of T2DM patients who had begun using premix BIAsp 30 (NovoMix®30, Novo Nordisk A/S), basal insulin detemir (IDet [Levemir®, Novo Nordisk A/S, Denmark]), or bolus IAsp (NovoRapid®, Novo Nordisk A/S) in routine clinical practice for T2DM management. The objectives of this study were to evaluate the clinical safety and effectiveness of these insulin regimens in routine clinical use outside the Western countries.[31] This paper focuses on the Egyptian patients recruited across 14 centers (N=412) between June 2009 and March 2010. A total of 131 Egyptian patients were prescribed BIAsp 30, either once or twice daily.

In the study, BIAsp 30 was used in line with the licensed approval from the Egyptian local regulatory authority. The study drug was prescribed by physicians as part of normal clinical practice, was commercially available, and purchased according to local practice. The advising physician determined the choice of insulin, the starting dose, administration frequency, and any later changes to either frequency or dose. At the time of starting BIAsp 30, or thereafter, any changes to oral glucose-lowering drugs (OGLDs) were entirely at the discretion of the advising physician.

There were no defined study-related procedures; measurements were made by the treating physician team only as determined by normal clinical care. Thus, safety and effectiveness of therapy were determined from measurements made at usual clinic visits. Trial visits were defined as baseline, interim (around 12 weeks from baseline), and final (around 24 weeks from baseline) visit. Data were collected from the physicians' clinical notes and patients' recall and self-monitoring diary/meter at each visit, as available. This information was transferred to a standard case report form.

Patients:

Any patient with T2DM who was prescribed BIAsp 30 at the discretion of the physician was eligible. Patients who were treated with BIAsp 30 (alone or in combination) for more than 4 weeks prior to the study were excluded. Women who were pregnant, breast-feeding, or had the intention of becoming pregnant were excluded. Ethics committee approval was obtained from Egyptian authorities, and signed informed consent from all patients. Patients were free to withdraw from the study at any time. If they withdrew, the data collected was used for analysis until the point when consent was withdrawn. Safety events were reported as per the protocol. All investigators received specific training on the study protocol, case report form completion, informed consent, and safety reporting procedures.

Assessments and outcome measures:

The primary objective of this study was to evaluate the safety of BIAsp 30 based on the number of serious adverse drug reactions (SADRs), including major hypoglycemic events, related to BIAsp 30 from baseline to the final visit (Week 24). Secondary safety assessments included changes in the number of hypoglycemic events, nocturnal hypoglycemic events, adverse drug reactions (ADRs), and body weight at Week 24 compared to baseline. The ADRs were summarized by the number of events and the number and percentage of patients with adverse events.

Major hypoglycemic events were defined as events with severe central nervous system symptoms, consistent with hypoglycemia, which the patient was unable to self-treat, and accompanied by plasma glucose < 3.1 mmol/L or 56 mg/dL, or reversal of symptoms after either food intake or glucagon or intravenous glucose administration. Minor hypoglycemia was any event, with or without symptoms of hypoglycemia, with a plasma glucose reading < 3.1 mmol/L or 56 mg/dL that the patient was able to self-treat. Nocturnal hypoglycemia was defined as a symptomatic event consistent

with hypoglycemia that occurred during sleep between bedtime after the evening insulin injection and before getting up in the morning.

Efficacy assessments were the change from baseline to Week 24 in HbA_{1c}, FPG, PPPG, body weight, QoL, systolic blood pressure (SBP), and lipid profile (total cholesterol, triglycerides, high-density lipoprotein [HDL] cholesterol, and low-density lipoprotein [LDL] cholesterol). Health-related QoL was assessed using the EQ-5D questionnaire at baseline and after 24 weeks of therapy with BIAsp 30. Local laboratories were used for all laboratory measurements and were subject to local standardization and quality control procedures.

Statistical Methods:

Statistical analyses were performed for the entire cohort (both insulin-naïve patients and prior insulin users). Only descriptive analyses were performed for insulin-naïve patients and prior insulin users. Concurrent OGLD use was allowed throughout the study period.

Analyses of all safety and efficacy variables were performed using any patient enrolled in the study with the data relevant to that analysis. Continuous variables were summarized using descriptive statistics and discrete variables were summarized using frequency tables (n, %). All statistical analyses were two-sided, using a pre-specified 5% significance level, unless otherwise stated.

For the change from baseline in hypoglycemia, the percentage of patients reporting at least one event was analyzed using Fisher's exact test. The change from baseline in HbA1c, FPG, PPPG, body weight, blood lipids, QoL, and SBP was analyzed using paired t-test.

All data were analyzed by Novo Nordisk using SAS (Version 9.1.3).

Results:

Study patients:

Of the 412 patients enrolled from Egypt, a total of 131 (32%) patients were included in the BIAsp 30 treatment group and these data are presented here.

Patient characteristics and prior OGLD use in Egyptian patients treated with BIAsp 30 are presented for the entire cohort and by prior insulin therapy (insulin-naïve patients or prior insulin users) in Table I. Prior to study enrolment, 46 (35.1%) patients were on OGLDs alone, 35 (26.7%) patients were on OGLD + insulin therapy, 49 (37.4%) patients were on insulin only, and 1 (0.8%) patient received no medication for T2DM. The OGLDs most commonly used in the insulin-naïve group prior to study enrolment included sulphonyl urea in 44 (95.7%) patients and metformin in 33 (71.7%) patients. Among prior insulin users, the OGLDs most commonly used prior to study enrolment were metformin in 26 (74.3%) patients and sulphonyl urea in 21 (60.0%) patients. In the insulin-naïve group, the majority of patients used 2 OGLDs (24 patients [52.2%]) while in the prior insulin user group, the majority of patients used 1 OGLD (21 patients [60.0%]) prior to study enrolment.

More males (67.2%) than females (32.8%) were enrolled in the study. In the entire cohort, the average age was 52.4 years, mean BMI was 30.5 kg/m^2, and mean diabetes duration was 11.6 years. The mean baseline HbA_{1c} was 9.5% in the entire cohort, 9.8% in the insulin-naïve patients, and 9.4% in the prior insulin users (Table I).

The most frequent reasons given for change in therapy by the treating physicians were improvement of glycemic control (95%), patient dissatisfaction with current therapy (34%), and reduction in plasma glucose variability (34%).

A total of 11 (8.4%) patients withdrew from the study, with 7 (5.3%) patients withdrawing due to failure to maintain contact with their physician and the remaining 4 (3.1%) for a variety of other reasons, while 120 (91.6%) patients completed the study. No withdrawals due to ADRs occurred.

Entire cohort and by prior insulin usage:

Blood glucose lowering and insulin dose:

In the entire cohort, the total daily insulin dose at baseline was 54.9 ± 21.6 U/day, which had been titrated up to 60.8 ± 25.2 U/day at 24 weeks. For insulin-naïve patients, the total daily insulin dose of 44.7 ± 13.8 U/day at baseline was titrated up to 48.5 ± 15.9 U/day at 24 weeks. In prior insulin users, the pre-study insulin dose was 53.5±26.2 U/day, total starting insulin dose was 60.6±23.1 U/day, and total insulin dose at 24 weeks was 67.6 ± 26.8 U/day.

In the entire cohort, the majority of patients were on BIAsp 30 twice daily at baseline and

24 weeks (91.6% and 85.0%, respectively), followed by patients on BIAsp 30 thrice daily (6.1% and 11.7%, respectively), and BIAsp 30 once daily (2.3% and 3.3%, respectively).

Blood glucose control improved markedly and statistically significantly between baseline and Week 24 in the entire cohort (Table II: HbA_{1c} -1.9%, FPG -83.4 mg/dL, PPPG -119.9 mg/dL, all p<0.001). Based on descriptive data, clear improvements in blood glucose control were noted in both insulin-naïve patients and prior insulin users (Table II). The HbA_{1c}, FPG, and PPPG levels from baseline to Week 24 are presented in Figures 1 to 3 for the entire cohort and by pre-study therapy.

The percentage of patients achieving an HbA_{1c} < 7.0% increased from 1.8% at baseline to 22.8% at Week 24.

Hypoglycemia:

The reported rate of all hypoglycemic episodes in the 4 weeks before study visits differed for insulin-naïve patients and prior insulin users (Table III). No major hypoglycaemic events were reported in the entire cohort during the study.

In the insulin-naïve patients, the reported rate of overall hypoglycemia increased from 0.28 to 1.81 events/patient-year. In the prior insulin users, the reported rate of overall hypoglycemia decreased from 10.21 to 2.70 events/patient-year after 24 weeks.

For insulin-naïve patients, the rate of minor hypoglycemic events increased from 0.28 to 1.81 events/patient-year, while no nocturnal hypoglycemia was reported during the study. In the prior insulin users, the incidence of minor and nocturnal hypoglycemic events decreased from 9.29 to 2.70 events/patient-year and 3.40 to 0.34 events/patient-year, respectively, during the study period (Table III). Hypoglycemic events (per patient-year) in the entire cohort from baseline to Week 24 are presented in Figure 4.

Body weight, blood lipids and blood pressure control:

In the entire cohort, there was no statistically significant change in mean body weight (Table II). Marginal changes in body weight occurred in insulin-naïve patients (-0.4 kg) and prior insulin users (+0.4 kg).

Total cholesterol levels were reduced in the entire cohort from a mean of 5.6 mmol/L to 4.7 mmol/L after 24 weeks (Table II, -0.9 mmol/L, p<0.001). Mean LDL cholesterol levels fell from 3.5 mmol/L to 2.9 mmol/L after 24 weeks (-0.5 mmol/L, p<0.001). A significant reduction was also seen in triglyceride levels (-0.5 mmol/L, p<0.001). There was a marginal non-significant increase in HDL cholesterol levels during the study (+0.1mmol/L). Overall, improvements in the lipid profile were observed in both insulin-naïve patients (except HDL cholesterol) and prior insulin users (Table II). Mean SBP fell significantly in the entire cohort, from 138.0 to 130.4 mmHg after 24 weeks of BIAsp 30 treatment (-7.6 mmHg, p<0.001). Consistent reductions in the SBP levels were noted in both insulin-naïve patients and prior insulin users (Table II).

ADRs, SADRs and serious adverse events:

No ADRs, SADRs or deaths were reported in the Egyptian BIAsp 30 treatment group. Only one patient reported one SAE (0.02 events/patient-year) 'foot ulcer' in the system organ class 'skin and subcutaneous tissue disorders' (neuropathic ulcer) in the prior insulin user group.

Quality of life assessments:

QoL was evaluated based on the following parameters: anxiety/depression, mobility, pain/discomfort, self-care, usual activity, and current health state. For the entire cohort, significant improvements in the QoL were reported after 24 weeks (change in visual analog scores* = +15.9 ± 15.8, p<0.001). Based on descriptive data, QoL improved in both insulin-naïve patients and prior insulin users (change in visual analog scores = +16.6 ± 13.0 and +15.5 ± 17.3, respectively) (Table IV). Pain was most improved in the entire cohort and insulin-naïve patients, while anxiety was most improved in the prior insulin users (Table V).

*An individual's current health-related QoL state was measured by a standard vertical 20 cm visual analog scale (VAS) in the questionnaire. The EQ-5D VAS score ranges from 0 (worst imaginable health) to 100 (best imaginable health). Each health-state dimension can be converted to a single utility value using an EQ-5D value set, the UK VAS set in the A_1chieve study, and are anchored by '1.00' representing full health and '0.00' representing the state " dead ".

Table I: Patient characteristics for the entire cohort and by pre-study therapy – Egyptian data

	Entire Cohort	Insulin-naïve	Prior insulin users
n (%)	131 (100)	47 (35.9)	84 (64.1)
Sex, M/F (%)	67.2/32.8	70.2/29.8	65.5/34.5
Age[a], years	52.4 (8.9)	52.2 (7.7)	52.5 (9.6)
Body weight[a], kg	88.7 (14.4)	88.4 (14.6)	88.9 (14.4)
BMI[a], kg/m²	30.5 (4.9)	30.0 (5.0)	30.8 (4.9)
Diabetes duration[a], years	11.6 (6.4)	9.5 (5.1)	12.8 (6.8)
HbA_{1c}[a], %	9.5 (1.6)	9.8 (1.8)	9.4 (1.5)
Prior OGLDs, n (%)			
Metformin	59 (72.8)	33 (71.7)	26 (74.3)
Sulfonylureas	65 (80.2)	44 (95.7)	21 (60.0)
Thiazolidinediones	15 (18.5)	14 (30.4)	1 (2.9)
One/two/>two	31 (38.3)/ 38 (46.9)/ 12 (14.8)	10 (21.7)/ 24 (52.2)/ 12 (26.1)	21 (60.0)/ 14 (40.0)/ -

[a]Data are mean (SD)

Table II: Glucose control and body weight for the entire cohort and by pre-study therapy at baseline and after 24 weeks of BIAsp 30 therapy – Egyptian data

		Entire Cohort	Insulin-naïve*	Prior insulin users*
Insulin dose, U/day	N	131	47	84
	Pre-study	53.5 (26.2)	-	53.5 (26.2)
	Baseline	54.9 (21.6)	44.7 (13.8)	60.6 (23.1)
	Week 24	60.8 (25.2)	48.5 (15.9)	67.6 (26.8)
HbA_{1c}, %	N	95	37	58
	Baseline	9.5 (1.6)	9.8 (1.8)	9.4 (1.5)
	Week 24	7.6 (1.2)	7.4 (0.9)	7.8 (1.3)
	Change, p	-1.9 (1.6), <0.001	-2.4 (1.5)	-1.6 (1.6)
FPG, mg/dL	N	119	43	76
	Baseline	212.6 (71.3)	221.3 (66.5)	207.7 (73.8)
	Week 24	129.2 (41.4)	122.3 (39.6)	133.1 (42.2)
	Change, p	-83.4 (73.4), <0.001	-99.0 (78.0)	-74.6 (69.7)
PPPG, mg/dL	N	118	43	75
	Baseline	290.0 (82.4)	303.3 (73.3)	282.4 (86.7)
	Week 24	170.1 (50.8)	164.2 (59.0)	173.5 (45.6)
	Change, p	-119.9 (92.2), <0.001	-139.1 (96.1)	-108.9 (88.7)
Body weight, kg	N	113	40	73
	Baseline	89.1 (14.4)	89.0 (14.6)	89.2 (14.4)
	Week 24	89.2 (13.8)	88.6 (13.8)	89.6 (13.8)
	Change, p	0.1 (4.0), 0.773	-0.4 (3.4)	0.4 (4.3)
SBP, mmHg	N	120	43	77
	Baseline	138.0 (16.6)	137.3 (20.2)	138.3 (14.4)
	Week 24	130.4 (16.1)	128.7 (10.9)	131.3 (18.4)
	Change, p	-7.6 (19.2), <0.001	-8.6 (19.6)	-7.0 (19.1)
Total cholesterol mmol/L	N	58	16	42
	Baseline	5.6 (1.2)	5.9 (0.7)	5.4 (1.3)
	Week 24	4.7 (1.2)	4.4 (1.5)	4.8 (1.1)
	Change, p	-0.9 (1.5), <0.001	-1.5 (1.6)	-0.7 (1.4)
Triglyceride, mmol/L	N	58	16	42
	Baseline	2.3 (1.0)	2.7 (1.2)	2.1 (0.8)
	Week 24	1.8 (0.6)	1.7 (0.6)	1.8 (0.6)
	Change, p	-0.5 (1.0), <0.001	-1.0 (1.1)	-0.3 (0.9)
HDL cholesterol, mmol/L	N	41	9	32
	Baseline	1.1 (0.3)	1.2 (0.5)	1.1 (0.2)
	Week 24	1.2 (0.4)	1.1 (0.1)	1.2 (0.4)
	Change, p	0.1 (0.4), 0.233	-0.1 (0.5)	0.1 (0.4)
LDL cholesterol, mmol/L	N	41	9	32
	Baseline	3.5 (1.1)	4.0 (0.5)	3.3 (1.1)
	Week 24	2.9 (0.8)	3.3 (0.6)	2.8 (0.8)
	Change, p	-0.5 (0.9), <0.001	-0.8 (0.9)	-0.5 (0.9)

Data are mean (SD) unless otherwise stated
*only descriptive data is presented for insulin-naïve patients and prior insulin users

Table III : Hypoglycemic events for the entire cohort and by pre-study therapy at baseline and after 24 weeks of BIAsp 30 therapy – Egyptian data

		Entire Cohort	Insulin-naïve*	Prior insulin users*
Overall	Baseline	6.65/19.8	0.28/2.1	10.21/29.8
	Week 24	2.38/10.8	1.81/7.0	2.70/13.0
	[a]p	0.0559	-	-
Minor	Baseline	6.05/19.1	0.28/2.1	9.29/28.6
	Week 24	2.38/10.8	1.81/7.0	2.70/13.0
	[a]p	0.079	-	-
Nocturnal	Baseline	2.18/9.2	0.00/0.00	3.40/14.3
	Week 24	0.22/1.7	0.00/0.00	0.34/2.6
	[a]p	0.0117	-	-
Major	Baseline	0.60/1.5	0.00/0.00	0.93/2.4
	Week 24	0.00/0.00	0.00/0.00	0.00/0.00
	[a]p	0.499	-	-

Data are presented as events per patient-year/percent with at least one event
[a]p-value is for difference in percent of patients with at least one event
*only descriptive data is presented for insulin-naïve patients and prior insulin users

Table IV: Quality of life at baseline and 24 weeks for patients treated with BIAsp 30 therapy – Egyptian data

		Entire Cohort	Insulin-naïve*	Prior insulin users*
EQ-VAS	N	116	42	74
	Baseline	65.8 (15.9)	66.9 (13.8)	65.2 (17.0)
	Week 24	81.7 (12.4)	83.6 (11.0)	80.7 (13.1)
	Change, p	15.9 (15.8), <0.001	16.6 (13.0)	15.5 (17.3)

Data are mean (SD) unless otherwise stated
*only descriptive data is presented for insulin-naïve patients and prior insulin users

Table V: Quality of life results for the entire cohort and by pre-study therapy at baseline and after 24 weeks of BIAsp 30 therapy – Egyptian data

		Entire Cohort	Insulin-naïve*	Prior insulin users*
Activity	Baseline	66 (55.0)	23 (53.5)	43 (55.8)
	Week 24	89 (74.2)	37 (86.0)	52 (67.5)
	p	0.0019	-	-
Anxiety	Baseline	68 (57.1)	26 (60.5)	42 (55.3)
	Week 24	90 (75.6)	34 (79.1)	56 (73.7)
	p	0.0025	-	-
Mobility	Baseline	78 (65.0)	30 (69.8)	48 (62.3)
	Week 24	92 (76.7)	37 (86.0)	55 (71.4)
	p	0.0468	-	-
Pain	Baseline	42 (35.3)	17 (39.5)	25 (32.9)
	Week 24	67 (56.3)	32 (74.4)	35 (46.1)
	p	0.0011	-	-
Self-care	Baseline	105 (87.5)	40 (93.0)	65 (84.4)
	Week 24	112 (93.3)	42 (97.7)	70 (90.9)
	p	0.1248	-	-

Data are mean (SD) unless otherwise stated
*only descriptive data is presented for insulin-naïve patients and prior insulin users

Table VI: Demographic and baseline characteristics of patients on BIAsp 30 therapy – Regional data

	Egypt	North Africa	Middle East + Gulf	Global study
n (%)	131 (100)	1545 (100)	8046 (100)	40917 (100)
Sex, M/F (%)	67.2/32.8	42.9/57.1	53.2/46.8	56.1/43.9
Age[a], years	52.4 (8.9)	57.9 (11.8)	54.1 (11.5)	54.0 (11.8)
Body weight[a], kg	88.7 (14.4)	75.4 (13.4)	81.9 (15.4)	71.4 (13.8)
BMI[a], kg/m^2	30.5 (4.9)	28.1 (5.1)	29.8 (5.3)	26.6 (4.6)
Diabetes duration[a], years	11.6 (6.4)	11.2 (7.4)	10.2 (6.3)	7.6 (6.0)
HbA$_{1c}$[a], %	9.5 (1.6)	9.6 (1.9)	9.7 (1.7)	9.5 (1.7)
Prior OGLDs, n (%)				
Metformin	59 (72.8)	825 (76.2)	6126 (92.4)	27019 (82.0)
Sulfonylureas	65 (80.2)	597 (55.2)	4087 (61.6)	21532 (65.3)
Thiazolidinediones	15 (18.5)	20 (1.8)	1855 (28.0)	6154 (18.7)
One/two/>two	31 (38.3)/ 38 (46.9)/ 12 (14.8)	574 (53.0)/ 445 (41.1)/ 63 (5.8)	2208 (33.3)/ 2630 (39.7)/ 1793 (27.0)	10295 (31.2)/ 16453 (49.9)/ 6221 (18.9)
[a]Data are mean (SD)				

Table VII: Baseline and 24-week data for safety and effectiveness outcomes for BIAsp 30 therapy – Regional data.

		Egypt	North Africa	Middle East + Gulf	Global study
Insulin dose, U/day	n	131	1544	8040	40910
	Pre-study	53.5 (26.2)	43.8 (20.0)	50.4 (23.2)	40.5 (21.3)
	Baseline	54.9 (21.6)	40.7 (16.0)	45.8 (20.0)	32.7 (15.8)
	Week 24	60.8 (25.2)	49.5 (17.6)	56.3 (22.2)	36.8 (19.1)
HbA$_{1c}$, %	n	95	912	5829	27033
	Baseline	9.5 (1.6)	9.6 (1.9)	9.7 (1.7)	9.5 (1.7)
	Week 24	7.6 (1.2)	7.9 (1.3)	7.5 (1.1)	7.4 (1.0)
	Change, p	-1.9 (1.6), <0.001	-1.8 (1.9), <0.001	-2.2 (1.7), <0.001	-2.1 (1.7), <0.001
FPG, mg/dL	n	119	1082	5581	29884
	Baseline	212.6 (71.3)	213.1 (79.7)	210.1 (70.3)	197.0 (63.4)
	Week 24	129.2 (41.4)	146.4 (50.6)	130.2 (36.0)	128.8 (34.1)
	Change, p	-83.4 (73.4), <0.001	-66.7 (91.4), <0.001	-80.0 (69.7), <0.001	-68.3 (62.9), <0.001
PPPG, mg/dL	n	118	650	3658	20811
	Baseline	290.0 (82.4)	277.4 (87.2)	280.1 (85.1)	274.0 (77.7)
	Week 24	170.1 (50.8)	187.5 (62.5)	169.1 (48.0)	176.7 (51.7)
	Change, p	-119.9 (92.2), <0.001	-90.0 (102.6), <0.001	-110.9 (84.7), <0.001	-97.3 (80.5), <0.001
Body weight, kg	n	113	1198	5785	30194
	Baseline	89.1 (14.4)	75.3 (13.1)	82.0 (14.8)	71.6 (13.6)
	Week 24	89.2 (13.8)	76.7 (12.4)	82.1 (14.0)	71.9 (13.0)
	Change, p	0.1 (4.0), 0.773	1.3 (4.3), <0.001	0.1 (4.2), 0.271	0.3 (3.5), <0.001
SBP, mmHg	n	120	1086	6184	26247
	Baseline	138.0 (16.6)	132.5 (17.5)	135.8 (18.4)	134.5 (17.9)
	Week 24	130.4 (16.1)	130.5 (14.8)	128.9 (14.0)	127.8 (12.8)
	Change, p	-7.6 (19.2), <0.001	-2.0 (17.1), <0.001	-6.9 (17.6), <0.001	-6.8 (17.0), <0.001
Total cholesterol, mmol/L	n	58	520	4128	11011
	Baseline	5.6 (1.2)	4.8 (1.2)	5.3 (1.2)	5.3 (1.3)
	Week 24	4.7 (1.2)	4.6 (0.9)	4.7 (0.9)	4.8 (1.0)
	Change, p	-0.9 (1.5), <0.001	-0.2 (1.2), <0.001	-0.6 (1.2) , <0.001	-0.5 (1.2), <0.001
Triglycerides, mmol/L	n	58	527	4114	10996
	Baseline	2.3 (1.0)	1.6 (1.0)	2.2 (1.0)	2.1 (1.1)
	Week 24	1.8 (0.6)	1.5 (0.7)	1.8 (0.7)	1.8 (0.7)
	Change, p	-0.5 (1.0), <0.001	-0.1 (0.9), 0.012	-0.3 (1.0), <0.001	-0.3 (1.0), <0.001

HDL cholesterol, mmol/L	n	41	388	3793	9962
	Baseline	1.1 (0.3)	1.1 (0.4)	1.1 (0.3)	1.2 (0.4)
	Week 24	1.2 (0.4)	1.2 (0.4)	1.1 (0.3)	1.2 (0.4)
	Change, p	0.1 (0.4), 0.233	0.0 (0.5), 0.545	0.0 (0.4), <0.001	0.1 (0.4), <0.001
LDL cholesterol, mmol/L	n	41	364	3909	10085
	Baseline	3.5 (1.1)	2.9 (1.3)	3.2 (1.0)	3.1 (1.1)
	Week 24	2.9 (0.8)	2.8 (1.1)	2.8 (0.8)	2.8 (0.9)
	Change, p	-0.5 (0.9), <0.001	-0.2 (1.6), 0.043	-0.4 (1.1), <0.001	-0.4 (1.1), <0.001
Hypoglycaemia (event per patient-year/percent with event)					
Overall	Baseline	6.65/19.8	8.94/22.9	3.24/8.7	2.43/7.7
	Week 24	2.38/10.8	5.31/19.5	2.35/8.8	1.46/5.7
	[a]p	0.0559	0.0272	0.9306	<0.0001
Minor	Baseline	6.05/19.1	7.43/22.4	2.91/8.3	2.19/7.4
	Week 24	2.38/10.8	5.17/19.3	2.34/8.7	1.45/5.7
	[a]p	0.079	0.0465	0.3782	<0.0001
Nocturnal	Baseline	2.18/9.2	3.95/15.0	0.76/3.5	0.70/3.2
	Week 24	0.22/1.7	1.60/7.9	0.56/2.9	0.32/1.7
	[a]p	0.0117	<0.0001	0.0367	<0.0001
Major	Baseline	0.60/1.5	1.51/7.3	0.34/1.7	0.24/1.2
	Week 24	0.00/0.0	0.14/0.5	0.01/0.1	0.008/0.04
	[a]p	0.499	<0.0001	<0.0001	<0.0001

Data are mean (SD) unless otherwise stated

[a] p-value is for difference in percent of patients with at least one event

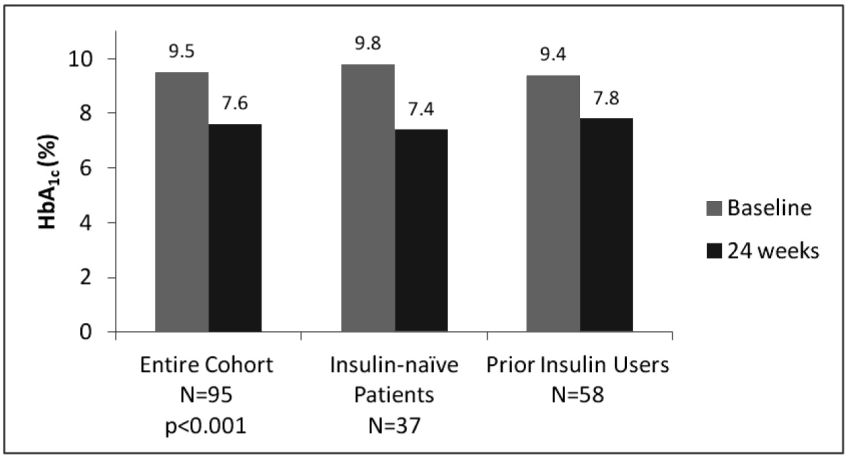


Figure 1: Glycated hemoglobin A_{1c} (HbA_{1c} %) for entire cohort and by pre-study therapy – Egyptian data

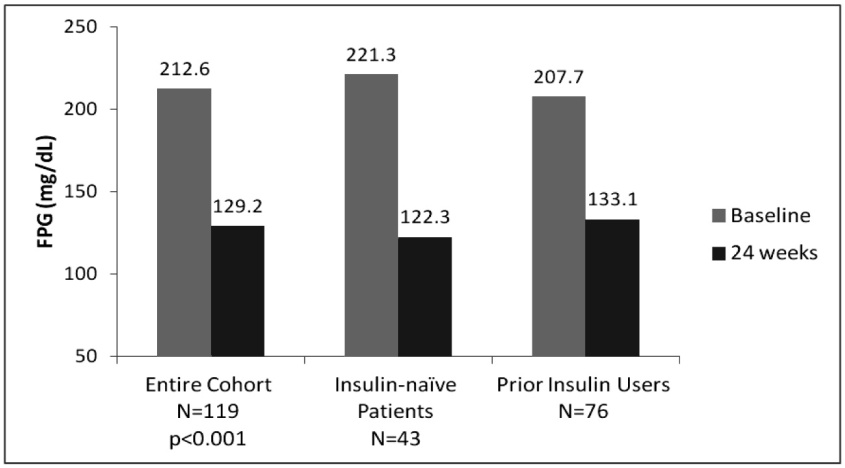


Figure 2: Fasting plasma glucose for entire cohort and by pre-study therapy – Egyptian data

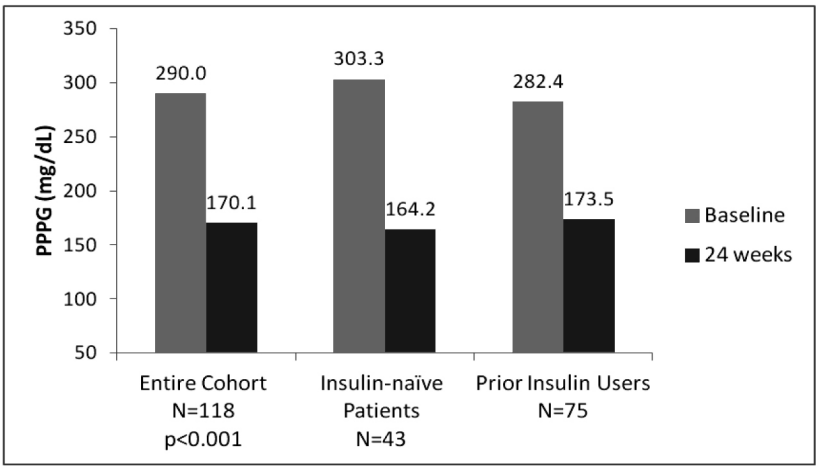
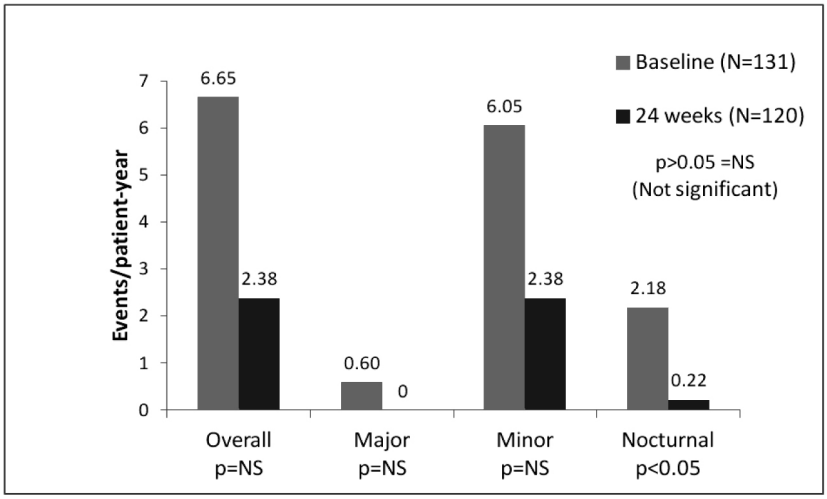


Figure 3: Postprandial plasma glucose for entire cohort and by pre-study therapy – Egyptian data



p-value is for the difference in percent of patients with at least one event.

Figure 4: Hypoglycemic events per patient-year in the entire cohort – Egyptian data

Discussion:

This sub-analysis demonstrated the safety and effectiveness of BIAsp 30 in Egyptian T2DM patients who initiated insulin analogue therapy or switched from prior insulin therapy. BIAsp 30 was well-tolerated and improved glycaemic control in all patients irrespective of the pre-study insulin therapy type. These results for BIAsp 30 were also demonstrated in a previous global study.[32]

The average age was lower in this Egyptian cohort as compared to the Middle East + Gulf, North Africa, and global A_1chieve data (Table VI). As reported in Table VI, body weight was the highest in Egypt while BMI was similar across regions. The average duration of diabetes was also longer in Egypt, Middle East + Gulf, and North Africa compared to the global A_1chieve data. Baseline HbA_{1c} in Egypt was comparable to the global A_1chieve data but it was lower than the Middle East + Gulf and North African regions.

Intensifying insulin therapy in T2DM patients in order to achieve target HbA_{1c} levels is often associated with an increased frequency of hypoglycaemic events.[33] However, in this sub-analysis, treatment with BIAsp 30 in Egypt resulted in the greatest decrease in overall hypoglycaemia rates compared to the Middle

East + Gulf, North Africa, and global A1chieve data (Table VII). No major hypoglycaemia was reported in the entire Egyptian cohort. As expected with insulin therapy initiation, insulin-naïve patients initiating BIAsp 30 reported an increase in overall hypoglycaemia owing to an increase, primarily, in the incidence of minor hypoglycaemic events. Overall, minor, and nocturnal hypoglycaemia decreased in prior insulin users switching to BIAsp 30 in the Egyptian cohort. Similar results have been reported in the Middle East + Gulf, North Africa, and global A1chieve data (Table VII). These results are in accordance with the PRESENT study on BIAsp 30.[34]

The average baseline HbA_{1c} level of 9.5% in this cohort reflects the suboptimal glycaemic control that was evident in the global A1chieve data as well. BIAsp 30 therapy resulted in statistically significant improvements in HbA_{1c}, FPG, and post-breakfast PPPG in this Egyptian cohort. These improvements were reported in both insulin-naïve patients and prior insulin users. Clear reductions in these glycaemic parameters have also been reported in the global A1chieve data [30] and in the IMPROVE study.[24] The reduction in HbA_{1c} also resulted in an increase from 1.8% at baseline to 22.8% at Week 24 in the number of patients achieving HbA_{1c} target levels <7.0%.

As seen in the Egyptian cohort, while high baseline HbA_{1c} levels were a concern, it is encouraging that treatment with BIAsp 30 was able to bring them in good control in short term, making it an effective treatment option for poorly controlled diabetes patients. These findings further corroborate a previous study which showed that insulin therapy with BIAsp 30 effectively achieved HbA_{1c} targets in T2DM subjects poorly controlled on OGLDs.[35] BIAsp 30 therapy in this study was associated with good FPG and PPPG control in the Egyptian cohort. Thus, BIAsp 30 is an attractive option for patients requiring insulin therapy addressing both prandial and basal insulin requirements.

Furthermore, improvements in cardiovascular factors may be instrumental in reducing the risk of micro- and macrovascular complications associated with T2DM.[36-38] Despite the long duration of diabetes observed in this cohort, SBP levels were observed to decrease along with improvements in the lipid profile in both insulin-naïve patients (except HDL cholesterol) and prior insulin users. These results are consistent with data from Middle East + Gulf, North Africa, and the global A1chieve cohort (Table VII). Improvements in lipids and SBP could also indicate better self-management behaviours due to adequate patient education.

It has been established that insulin therapy is often associated with unwarranted weight gain.[39] However, following BIAsp 30 therapy there was no statistically significant change in weight reported for this Egyptian cohort and the Middle East + Gulf region. The North African region reported the largest statistically significant overall weight gain (+1.3 kg, Table VII). Previous studies on BIAsp 30 indicated marginal weight gain[24, 40] to unchanged body weight.[41]

BIAsp 30 therapy also had a significant impact on QoL as evidenced by clear improvements in the EQ-5D questionnaire recorded in the Egyptian cohort, similar to the reported improvements in the Middle East + Gulf, North Africa, and global A1chieve data [30] and the IMPROVE study.[42] In this study, the QoL improved irrespective of pre-study therapy type in the Egyptian cohort. The greatest improvements were noted in pain in the entire cohort and insulin-naïve patients, while anxiety was most improved in the prior insulin users.

In summary, initiating or switching to BIAsp 30 therapy in routine clinical care in Egypt improved glycaemic control with no major hypoglycaemia. Furthermore, significant improvements in QoL were also observed in this cohort. These benefits make BIAsp 30 an effective and convenient tool in T2DM management.

Conflict of interest

Sherif Waguih and Mohamed Fahmy are employed by Novo Nordisk.

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Original Article

A Pilot Study of 25-Hydroxy Vitamin D in Egyptian Diabetic Patients with Diabetic Retinopathy.

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Abstract:

Background: Many cellular, preclinical and observational studies support a role of vitamin D in the pathogenesis of type 2 diabetes. Vitamin D is suggested to be an inhibitor of angiogenesis. A growing body of evidence suggests an association between vitamin D inadequacy and diabetic microvascular complications e.g. diabetic nephropathy but little data are available about the association with diabetic retinopathy (DR). **Objective:** To explore a hypothesized association between 25 (OH) vitamin D level and DR in patients with type 2 diabetes mellitus and to evaluate for the presence of any relation between 25 (OH) vitamin D level and different stages of DR. **Research Design and Methods:** This pilot study was conducted on 50 type 2 diabetic patients divided into two groups: group (I) including 25 patients with diabetic retinopathy and group (II) including 25 patients without diabetic retinopathy. 50 healthy volunteers matched by age and sex were selected as a control group. The patients were selected from Ain Shams University Hospital from April to June 2010. Fasting blood sugar, HbA1c, renal functions, liver functions, lipid profile, serum calcium, serum phosphorous, intact parathyroid hormone (iPTH) and serum 25 hydroxyvitamin D_3(25(OH)D_3) levels were done to all participants in the study. A complete ophthalmic and fundus examination was done for all patients. **Results:** Mean 25(OH) vitamin D level was lower in type 2 diabetic cases than in control group (21.48±5.24 ng/ml vs. 45.79±11.08ng/ml) (P<0.01). Mean 25(OH) vitamin D level was lower in type 2 diabetic cases with DR than type 2 diabetic cases without DR (18.78±4.62 ng/ml vs. 24.17±5.80 ng/ml) (P<0.01). Patients with PDR have the lowest mean 25(OH) vitamin D level compared to patients with moderate NPDR and severe NPDR (13.53±3.36 ng/ml vs. 21.57±5.38 ng/ml and 16.20±4.00 ng/ml) (P<0.01). 25(OH) vitamin D level was inversely correlated with age, duration of type 2 diabetes mellitus, degree of microalbuminuria, fundus findings, BMI, SBP, DBP, glycemic parameters (including FBS and HbA_1c%), urinary ACR, total cholesterol, triglycerides, LDL-C (P<0.01) and iPTH level (P<0.05). 25(OH) vitamin D level was positively correlated with GFR, HDL-C and total calcium level (P<0.01) among all studied groups. **Conclusions:** There was a significant reduction of 25 (OH) vitamin D level in patients with type 2 diabetes and more so in patients with DR. Moreover, this study demonstrated a negative correlation between 25 (OH) vitamin D level and severity of diabetic retinopathy among patients with type 2 diabetes mellitus. These findings reflect a possible causality relationship between vitamin D deficiency and the development and progression of diabetic retinopathy. We recommend to validate our results in larger trials and to study the effect of vitamin D supplements on the prevention of DR.

Keywords: 25-Hydroxy Vit D - Diabetes - Retinopathy.

Introduction:

Diabetic retinopathy is a highly specific vascular complication and a sight-threatening problem related to diabetes. It is characterized by gradually progressive alterations in the retinal microvasculature, leading to retinal nonperfusion, increased vascular permeability and pathologically intraocular proliferation of retinal vessels[(1)].

The cause of complications in the diabetic state has been a subject of intense research for over half of a century. However, two major clinical trials, DCCT and UKPDS, established the relationship of poor glycemic control to diabetic retinopathy[(2,3)]. The precise relationship of other factors to diabetic complications is still not clear.

Abbreviations: 25(OH) D_3, 25(OH) vitamin D_3; DR, diabetic retinopathy; FBS, fasting blood sugar; GFR, glomerular filtration rate; HbA_1c%, glycated hemoglobin; HDL-C, high density lipoprotein cholesterol; iPTH, intact parathyroid hormone; LDL-C, low density lipoprotein cholesterol; NPDR, non proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; urinary ACR, urinary albumin creatinine ratio.

Vitamin D is now recognized as an important prohormone in health and disease[4]. Recently, vitamin D has sparked widespread interest in the pathogenesis and prevention of diabetes[5]. Diaz and his colleagues[6] demonstrated an association between vitamin D deficiency and vitamin D insufficiency with nephropathy in individuals with diabetes even after controlling for factors such as race/ethnicity, presence of hypertension and use of ACE inhibitors or ARBs. The high prevalence of vitamin D deficiency and vitamin D insufficiency in individuals with diabetes suggest that studies to further describe the role of vitamin D as a possible risk marker or risk factor in diabetic nephropathy are needed to evaluate the impact of maintaining an adequate level of vitamin D on the progression of diabetic nephropathy.

Taverna and his colleagues[7] demonstrated that the vitamin D receptor (VDR) is extensively expressed in retina. However, little data are available about the association with DR. The aim of the present study was to examine the potential relationship between serum 25 (OH) vitamin D level and DR and to evaluate for the presence of any relation with different stages of DR.

Material and Methods:

Study Design

This study comprised 100 subjects. 50 type 2 diabetic patients divided into two groups: group (I) including 25 patients with diabetic retinopathy and group (II) including 25 patients without diabetic retinopathy. 50 healthy volunteers matched by age and sex were selected as a control (group III). The patients were selected from Ain Shams University Hospital from April to June 2010. This study was approved by the Ethical Committee of Ain Shams University. An informed detailed consent was taken from all subjects before the start of the study.

Methodology:

Calculation of BMI and measurement of arterial blood pressure were performed for all subjects. The estimated glomerular filtration rate (GFR) was calculated using the Modification of Diet in Renal Disease formula (MDRD). Complete ophthalmic examination and fundus examination were done for all patients. Based on their ophthalmic findings, the type 2 diabetic patients were divided into two groups: group (I) including 25 patients with DR and group (II) including 25 patients without DR. Fluorescein angiography was done for patients of group (I) and accordingly the patients were further classified into 3 subgroups: moderate nonproliferative diabetic retinopathy (moderate NPDR), severe nonproliferative diabetic retinopathy (severe NPDR) and proliferative diabetic retinopathy (PDR). Each subgroup is then divided according to presence or absence of clinically significant macular edema (CSME). When the diabetic retinopathy was asymmetric, the subject was assigned to the group corresponding to the eye with the worse retinopathy findings.

Biochemical Measurements:

Subjects were asked to faste for 8-12 hours over night. Venous blood sample was withdrawn for analyzing the fasting blood sugar, serum creatinine, serum calcium, serum phosphorus, AST, ALT, serum albumin, prothrombin time, HbA1c, lipid profile, 25 (OH) vitamin D and iPTH. Serum 25 (OH) vitamin D level was measured by using Immundiagnostik direct ELISA kit[8]. Levels of serum 25(OH) vitamin D_3 >30 ng/ml were sufficient, levels from 12 ng/ml to 30 ng/ml were insufficient, while levels <12 ng/ml were deficient[9,10]. iPTH was measured by using Calbiotech iPTH direct ELISA kit[11]. A morning urine sample was collected from each subject for complete urine analysis and measurement of urinary albumin/creatinine ratio. Values of urinary albumin/creatinine ratio between 30-300µg/mg and over 300µg/mg in spot urine were considered as microalbuminuria or macro albuminuria respectively.[12]

Exclusion Criteria:

Exclusion criteria included type 1 diabetic patients, type 2 diabetic patients who are receiving vitamin D or VDR agonist or calcium supplements or any medications that may affect vitamin D level (as antiseizures, glucocorticoids, orlistat, ketoconazole or cholestyramine), pregnant women, smokers, patients with acute hyperglycemic states, patients with chronic liver disease, patients with serum creatinine >1.5 mg/dl in male or >1.4 mg/dl in female, patients with GFR < 90 ml / min/1.73 m^2 and cancer patients. Information on age, duration of type 2 diabetes, medical history and drug history was obtained from patients.

Statistical Analysis:

All data processing and statistical analysis were done with SPSS version 15. Description of data was in the form of mean ± standard deviation for quantitative variables and percentage for qualitative variables. Student's t-test, ANOVA test and Chi-square (X^2) tests were used to compare variables between groups. Correlation-coefficient test (r-test) was used to rank different variables against each other either positively or negatively. The significance of the results was assessed in the form of P-value differentiated into: non-significant when P-value >0.05, significant when P-value ≤0.05 and highly significant when P-value ≤ 0.01.

Results:

Comparison of different variables between the studied groups (Table 1) revealed that there is no statistical difference between studied groups as regard age, body mass index, serum creatinine, glomerular filtration rate, total calcium or serum phosphorous levels. Type 2 diabetic patients with diabetic retinopathy had longer duration of the disease than those without retinopathy with a statistically significant difference (P<0.05). there was a statistically highly significant difference between the studied groups as regard systolic and diastolic blood pressures, parameters of glycemic control including fasting blood sugar and HbA1C%, urinary ACR, total cholesterol, triglycerides, LDL-C and iPTH being higher in type 2 diabetic patients without DR than control group and the highest in type 2 diabetic patients with DR. Additionally, HDL-C was lower in type 2 diabetic patients without DR than control group and the lowest in type 2 diabetic patients with DR with a highly significant statistical difference. 25(OH) vitamin D level was lower in type 2 diabetic cases with DR (n = 25) than type 2 diabetic cases without DR (n = 25) (18.78±4.62 ng/ml versus 24.17±5.80 ng/ml) (P<0.01).

Correlation study of 25(OH) vitamin D with different variables (Table 2) revealed that 25(OH) vitamin D level was inversely correlated with age, duration of type 2 diabetes mellitus, stages of diabetic nephropathy, fundus findings, BMI, SBP, DBP, glycemic parameters, urinary ACR, total cholesterol, triglycerides, LDL-C (P<0.01) and iPTH level (P<0.05) among type 2 diabetics. 25(OH) vitamin D level was positively correlated with GFR, HDL-C and total calcium level (P<0.01) among all studied groups.

Comparison of different variables between different grades of DR (Table 3) revealed that patients with PDR have the highest mean serum creatinine (0.86±0.11mg/dl), urinary ACR (136.27±25.73 µg/mg) and iPTH (60.43 ± 15.08 pg/ml) and the lowest mean glomerular filtration rate (93.67±7.55 ml/min/ 1.73m^2) (P<0.05). Patients with PDR have the lowest mean 25(OH) vitamin D level compared to patients with moderate NPDR and severe NPDR (13.53±3.36 ng/ml versus 21.57±5.38 ng/ml and 16.20±4.00 ng/ml respectively) (P<0.01) (Table 3). Type 2 diabetic patients with DR associated with CSME had a lower mean serum 25(OH) vitamin D_3 level compared to those without CSME (16.60±3.86 ng/ml versus 22.67±5.06 ng/ml) (P<0.01).

Table (1): Descriptive data and comparison of different variables between the studied groups

	Group I (type 2 diabetics with diabetic retinopathy) (N=25) Mean±SD	Group II (type 2 diabetics without diabetic retinopathy) (N=25) Mean±SD	Group III (control) (N=50) Mean ±SD	P
Age (years)	53.80 ± 8.67	53.08 ± 7.77	51.60 ± 6.85	>0.05
Duration of type 2 diabetes mellitus (years)	17.96 ± 4.08	15.44 ± 3.54	-	<0.05*
Body mass index (Kg/m²)	28.85 ± 3.90	27.93 ± 3.89	27.02 ± 3.06	>0.05
Systolic blood pressure (mmHg)	140.60 ± 13.10	131.40 ± 15.11	121.30 ± 5.23	<0.01**
Diastolic blood pressure (mmHg)	89.60±7.90	81.98 ± 4.68	79.70 ± 4.09	<0.01**
Fasting blood sugar (mg/dl)	252.60 ± 50.50	210.08 ± 51.40	83.18 ± 10.59	<0.01**
Glycated hemoglobin (HbA1c %)	10.01 ± 1.82	8.24 ± 1.76	4.82±0.47	<0.01**
Serum creatinine (mg/dl)	0.80 ± 0.08	0.85 ± 0.14	0.80 ± 0.06	>0.05
Glomerular filtration rate (ml/min/1.73 m^2)	109.32 ±18.77	110.44 ± 6.67	115.38 ± 18.47	>0.05
Urinary albumin/creatinine ratio (µg/mg)	124.44±30.32	86.07±20.80	12.84 ± 2.44	<0.01**
Total cholesterol (mg/dl)	250.64 ± 61.51	234.76±51.40	155.30 ± 19.18	<0.01**
Triglycerides (mg/dl)	178.48 ± 44.26	173.24±42.69	88.36 ± 21.19	<0.01**
Low density lipoprotein cholesterol (mg/dl)	175.52 ± 43.65	163.17 ± 40.09	86.89 ± 19.33	<0.01**
High density lipoprotein cholesterol (mg/dl)	39.44 ± 7.74	36.68 ± 6.61	50.86 ± 4.55	<0.01**
Total calcium (mg/dl)	9.28 ± 0.56	9.16 ± 0.56	9.26 ± 0.47	>0.05
Serum phosphorus (mg/dl)	3.59 ± 0.48	3.68 ± 0.46	3.69 ± 0.57	>0.05
Intact Parathyroid Hormone (pg/ml)	46.68 ± 11.26	41.21 ± 7.62	34.01 ± 8.03	<0.01**
25 (OH) vitamin D_3 (ng/ml)	18.78 ± 4.62	24.17 ± 5.80	45.79 ±11.08	<0.01**

* Significant difference. ** Highly significant difference.

Table (2): Correlation coefficient study of 25 (OH) vitamin D level with different variables

	Variables	r	P
25 (OH) vitamin D_3 (ng/ml)	Age (years)	- 0.52	<0.01**
	Duration of type 2 diabetes mellitus (years)	- 0.59	<0.01**
	Stages of diabetic nephropathy	-0.62	<0.01**
	Fundus findings	-0.40	<0.01**
	Body mass index (Kg/m²)	- 0.48	<0.01**
	Systolic blood pressure (mmHg)	- 0.73	<0.01**
	Diastolic blood pressure (mmHg)	- 0.69	<0.01**
	Fasting blood sugar (mg/dl)	- 0.84	<0.01**
	Glycated hemoglobin (HbA1c %)	- 0.75	<0.01**
	Serum creatinine (mg/dl)	- 0.16	>0.05
	Glomerular filtration rate (ml/min/1.73 m^2)	0.46	<0.01**
	Urinary albumin creatinine ratio (µg/mg)	- 0.82	<0.01**
	Total cholesterol (mg/dl)	- 0.71	<0.01**
	Triglycerides (mg/dl)	- 0.58	<0.01**
	Low density lipoprotein cholesterol (mg/dl)	- 0.71	<0.01**
	High density lipoprotein cholesterol (mg/dl)	0.47	<0.01**
	Total calcium (mg/dl)	0.61	<0.01**
	Serum phosphorus (mg/dl)	0.20	>0.05
	Serum albumin (gm/dl)	0.27	>0.05
	Intact Parathyroid Hormone (pg/ml)	- 0.33	<0.05*

Table (3): Comparison of different variables between different grades of diabetic retinopathy

	Moderate Non Proliferative Diabetic Retinopathy (N=15) Mean±SD	Severe Non Proliferative Diabetic Retinopathy (N=4) Mean±SD	Proliferative Diabetic Retinopathy (N=6) Mean ±SD	P
Age (years)	46.87 ± 2.88	55.25 ± 2.50	66.00 ± 3.74	<0.01**
Duration of type 2 diabetes mellitus (years)	14.80 ± 3.34	19.25 ± 1.50	25.00 ± 3.16	<0.01**
Body mass index (Kg/m²)	28.73 ± 3.76	27.08 ± 4.22	30.33 ± 4.19	>0.05
Systolic blood pressure (mmHg)	136.30 ± 13.69	142.50 ± 9.57	151.67 ± 7.53	<0.05*
Diastolic blood pressure (mmHg)	87.67 ± 7.99	86.25 ± 4.79	96.67 ± 5.16	<0.05*
Fasting blood sugar(mg/dl)	223.20 ± 33.57	273.00 ± 40.61	301.50 ± 38.71	<0.01**
Glycated hemoglobin (HbA1c %)	8.91 ± 0.94	10.63 ± 1.52	12.35 ± 1.26	<0.01**
Serum creatinine (mg/dl)	0.77 ± 0.05	0.78 ± 0.02	0.86 ± 0.11	<0.05*
Glomerular filtration rate (ml/min/1.73 m^2)	115.47 ±18.23	109.75 ± 22.23	93.67 ± 7.55	<0.05*
Urinary albumin/ creatinine ratio (µg/mg)	101.52 ± 25.06	107.35 ± 25.86	136.27 ± 25.73	<0.05*
Total cholesterol(mg/dl)	231.47 ± 50.84	258.50 ± 39.53	293.00 ± 36.27	<0.05*
Triglycerides (mg/dl)	159.27 ± 33.56	188.75 ±14.27	207.83 ± 41.62	<0.05*
Low density lipoprotein cholesterol (mg/dl)	158.49 ± 35.70	182.05 ± 38.59	213.27 ± 34.54	<0.05*
High density lipoprotein cholesterol (mg/dl)	41.13 ± 7.84	38.75 ± 3.59	31.90 ± 6.46	<0.05*
Total calcium (mg/dl)	9.39 ± 0.45	9.20 ± 0.87	9.03 ± 0.62	>0.05
Serum phosphorus(mg/dl)	3.59 ± 0.50	3.70 ± 0.34	3.50 ± 0.54	>0.05
Serum albumin (gm/dl)	4.17 ± 0.28	4.35 ± 0.25	4.43 ± 0.30	>0.05
Intact Parathyroid Hormone (pg/ml)	42.25 ± 10.56	47.65 ± 11.83	60.43 ± 15.08	<0.05*
25 (OH) vitamin D_3 (ng/ml)	21.57 ± 5.38	16.20 ± 4.00	13.53 ± 3.36	<0.01**

* Significant difference. ** Highly significant difference.

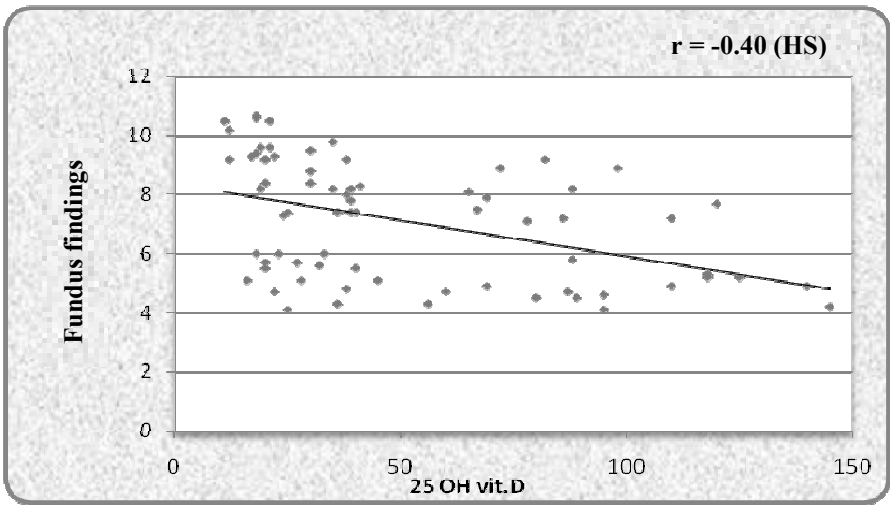

Figure (1): Correlation study of 25 (OH) vitamin D level and severity of diabetic retinopathy

Discussion:

Our results showed that mean serum 25(OH) vitamin D_3 level was lower in the 50 type 2 diabetic patients compared with the healthy control group with a highly significant statistical difference. This was in agreement with results of the previously conducted studies[13-16]. Some intrinsic factors unique to type 2 diabetes could be used as predictors for developing this state of hypovitaminosis. The significant higher age, BMI, glycemic indices and frequency of diabetic complications in the group with vitamin D insufficiency could represent the answer.

In our study we observed that type 2 diabetic patients with DR had the highest body mass index with no statistically significant difference if compared to type 2 diabetic patients without DR and statistically significant difference if compared with the healthy control counter partners. Dirani and colleagues[17] had presented several biological theories to explain the pathophysiological mechanisms of the association between higher BMI and DR. These include the potential involvement of platelet function, blood viscosity, aldose reductase activity and vasoproliferative parameters such as vascular endothelial growth factor. Apart from these parameters, lifestyle factors such as physical activity and weight loss provide some evidence to support this relationship[17].

In the present study, comparison between type 2 diabetic patients with diabetic retinopathy versus those without diabetic retinopathy revealed that patients with diabetic retinopathy had a significantly longer duration of type 2 diabetes mellitus, a significant higher systolic blood pressure and a highly significant higher diastolic blood pressure. Regarding parameters of glycemic control, there was a highly significant increase in FBS and HbA_{1C} in type 2 diabetic patients with diabetic retinopathy compared to type 2 diabetic patients without diabetic retinopathy. These results support findings of previous

studies[18,19]. It is presumed that duration of diabetes reflects total glycemic control and risk factor exposure over time. Additionally, DR can be affected by the hemodynamic changes induced by hypertension, such as impaired autoregulation and hyperperfusion. In addition, hypertension independent of hyperglycemia is known to upregulate the expression of vascular endothelial growth factor in retinal endothelial cells and ocular fluids, which can promote DR[19].

The results of the present study revealed that type 2 diabetic patients with diabetic retinopathy had a higher mean iPTH level compared to type 2 diabetic patients without diabetic retinopathy with a statistically significant difference. Patients with proliferative diabetic retinopathy had significantly the highest mean iPTH in comparison to other grades of diabetic retinopathy.

These results were in agreement with data from previous studies[20,21]. This finding may be explained by a compensatory mechanism to the low serum vitamin D3 concentrations. Parathyroid hormone excess can induce inflammatory cytokines which may play a role in the pathogenesis of proliferative DR[21].

The present study revealed that type 2 diabetic patients with severe NPDR have lower mean serum 25(OH) vitamin D level than type 2 diabetic patients with moderate NPDR and that type 2 diabetic patients with PDR have the lowest mean serum 25(OH) vitamin D level with statistically highly significant difference. This finding may suggest a permissive role of vitamin D deficiency in the pathogenesis of DR.

Additionally, vitamin D may play a role in the pathogenesis of diabetic retinopathy through its effects on the immune system. Inflammatory cytokines such as TNF-α, TNF-β, IL-6 and plasminogen activator inhibitor-1 are upregulated in patients with type 2 diabetes. It has been shown that vitamin D exerts an anti-inflammatory effect by decreasing the production of several proinflammatory cytokines such as IL-2, IL-6, IL-8, IL-12 and TNF-α as well as decreasing the proliferation of helper T-cells, cytotoxic T-cells and natural killer cells[22].

A recent study[23] found that vitamin D deficiency was associated with vascular endothelial dysfunction in middle aged and elderly adults. The authors concluded that this dysfunction was related to increased vascular endothelial cell expression of the proinflammatory transcription factor, nuclear factor κB.

Vitamin D may also contribute to diabetic retinopathy via angiogenesis mechanisms[14]. Albert and his colleagues[24] have shown that the active metabolite of vitamin D, calcitriol, was a potent inhibitor of both retinal neovascularization in vivo and retinal endothelial cell capillary morphogenesis in vitro.

In the current study we observed that patients with PDR have the highest mean serum creatinine, urinary ACR and iPTH levels as well as the lowest mean glomerular filtration rate and mean 25(OH) vitamin D level compared to patients with moderate and

severe NPDR. These findings may suggest that as renal function deteriorates, vitamin D synthesis decreases and stimulates iPTH which may play a direct role in the pathogenesis of DR and reveals the link between diabetic retinopathy and diabetic nephropathy.

Several complications in diabetic retinopathy such as macular edema and neovascularization are driven by VEGF production[25]. Therefore, vitamin D could exert its positive effect via calcitriol mediated VEGF reduction[24].

Vitamin D may also play a protective role through its effects on glycemic control and hypertension; both of them are significant risk factors for the development and progression of diabetic retinopathy[26].

There are several limitations to the current study. First, the cross sectional design of this study limits the ability to assess causality. It is not possible to determine whether the vitamin D insufficiency leads to diabetic retinopathy or if diabetic retinopathy leads to vitamin D insufficiency. Second, only one time point was recorded for the subjects in this study. It would be valuable to follow these patients with serial fundoscopic examinations and blood testing.

Conclusion:

There was a significant reduction of 25 (OH) vitamin D level in patients with type 2 diabetes and more so in patients with DR. Moreover, this study demonstrated a negative correlation between 25 (OH) vitamin D level and severity of diabetic retinopathy among patients with type 2 diabetes mellitus. These findings reflect a possible causality relationship between vitamin D deficiency and the development and progression of diabetic retinopathy. We recommend to validate our results in larger trials and to study the effect of vitamin D supplements on the prevention of DR.

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Original Article

Prevalence of Helicobacter Pylori in Diabetes Mellitus Patients with Non Ulcerative Dyspeptic Symptoms and its Relationship to Glycemic Control and Microalbuminuria.

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Abstract:

Background: Helicobacter pylori (HP) infection is an issue which is contentious and deserves further investigation. The relationship between DM and HP infection is controversial. **Objective:** The aim of this study was to determine the prevalence of Helicobacter pylori infection in type 2 diabetes mellitus patients with non ulcerative dyspepsia and to evaluate the relationship between H. pylori infection and the glycemic control and microalbuminuria. **Patients and methods:** This study included two groups of patients, **Group I:** Included 30 patients, non diabetic with dyspeptic symptoms and **Group II:** Included 30 patients, with type 2 diabetes with dyspeptic symptoms. All patients provided written informed consent before enrollment in the study. All patients were subjected to history taking, thorough clinical examination included neurological examination, laboratory investigation included CBC, fasting and postprandial plasma glucose, renal functions and liver function tests, HbAlc and microalbuminuria in 24 h collected urine and upper gastrointestinal endoscopy with biopsy specimens obtained from gastric antrum and corpus. H. pylori status was evaluated in each patient by both the rapid urease (CLO test) test and histopathological examination. **Result:** There were no statistical significant difference in the prevalence of H. pylori between the two groups by both histopathology and by CLO test (p=0.284 &0.118 respectively).Among the thirty diabetic patients, 17 patients were infected by H. pylori and 13 patients were not infected as shown by histopathological study. The mean fasting blood sugar (FBS), the prevalence of HbA1c > 7 , The prevalence of Microalbuminuria & the mean 24 hours protein were statistically significantly lower in H pylori negative patients than in H pylori positive patients (p=0.013,0.007 ,0.004&0,002 respectively). **Conclusion:** No statistical significant difference in the prevalence of H. pylori between diabetic and non diabetic patients. H pylori infection was significantly associated with poor glycemic control and high prevalence of microalbuminuria in diabetic patients.

Introduction:

Helicobactor pylori (HP) is a gram-negative, spiral-shaped pathogenic bacterium that specifically colonizes in the gastric epithelium and causes chronic gastritis, peptic ulcer disease, and/or gastric malignancies. [1, 2]

The infection induces an acute polymorph nuclear infiltration in the gastric mucosa. If the infection is not effectively cleared, this acute cellular infiltrate is gradually replaced by an immunologically mediated, chronic, predominantly mononuclear cellular infiltrate. [3] The latter is characterized by the local production and systemic diffusion of proinflammatory cytokines,[4] which may exert their effects in remote tissues and organic systems and result in extragastric manifestations. [5]

The prevalence of HP infection varies between countries; generally, the prevalence is about 30% in developed and up to 80% in developing countries. [6]

Diagnosis of HP can be achieved by taking biopsies by endoscopy. However, this procedure is invasive and might not give accurate results if colonization is patchy. [7] For population screening, serodiagnosis remains one of the methods of choice for detecting the prevalence of infection. [8–10] The technique of choice is currently enzyme-linked immunosorbent

assay because it is a simple, quick, and low-cost technique that permits immunoglobulin class-specific determinations.[11–18]

Diabetes mellitus (DM), a chronic disease marked by high levels of sugar in the blood, is common and increasing around the world.[19]

The relationship between DM and HP infection is controversial. According to some studies, there is a high prevalence of HP infection in patients with either Type 1 or Type 2 DM which is correlated with the duration of DM, the presence of dyspeptic symptoms, cardiovascular autonomic neuropathy, age, gender, BMI, blood pressure, fasting glucose, and the glycated hemoglobin levels (HbA1c).[20–22] In contrast, other studies showed that HP infection is not associated with DM. [20–22]

According to some data, there is no relationship between HP infection and diabetic complications, such as nephropathy, retinopathy, and/or microangiopathy while other data showed that virulent strains of HP, such as cytotoxin-associated gene CagA+, are associated with macroangiopathy, neuropathy, and microalbuminuria in Type 2 diabetic patients. [23, 26]

The aim of this study was to determine the prevalence of Helicobacter pylori infection in type 2 diabetes mellitus patients with non ulcerative dyspepsia and to evaluate the relationship between H. pylori infection and the glycemic control and microalbuminuria.

Patients and methods:

This study included two groups of patients:

Group I: Included 30 patients, non diabetic with dyspeptic symptoms.

Group II: Included 30 patients, with type 2 diabetes with dyspeptic symptoms.

Exclusion criteria included patients diagnosed previously to have H. pylori infection, patients receiving proton pump inhibitors or H_2 blockers before the endoscopy by 2 weeks, smokers, and patients with hypertension or any other cause of microalbuminuria or history of alcoholism or abuse of NSAI. All selected patients provided written informed consent before enrollment in the study.

All patients were subjected to the following: History taking and thorough clinical examination included neurological examination, laboratory investigation included; Routine laboratory investigation as CBC, fasting and postprandial plasma glucose, renal functions and liver function tests,[27&28] HbAlc,[28] Microalbuminuria in 24h collected urine [27] and upper gastrointestinal endoscopy with biopsy specimens obtained from gastric antrum and corpus. H. pylori status was evaluated in each patient by both the rapid urease test (CLO test) [29] and histopathological examination of endoscopic biopsies.[30] Data were collected, revised and transferred into statistical package for social science (SPSS/ version 10). Results were expressed as means and standard deviation. Statistical tests used in this study were (t.test, Fisher Exact test, Mann Whitney test, Monte Carlo test & Ci.square). A level of 5% was considered as the cutoff level of significance.

Result:

Our result shows that there were no statistical significant difference in the prevalence of H. pylori between the two groups by both histopathology and by CLO test (p=0.284 &0.118 respectively).Table (1) Also, the prevalence of H. pylori in both groups was statistically insignificantly higher by histopathology than by CLO test (p=0.093). Table (2)

Among the thirty diabetic patients, 17 patients were infected by H. pylori and 13 patients were not infected as shown by histopathological study. The mean age and the prevalence of obese were significantly higher in diabetic with H.pylori infection (58.88 ± 6.70 years & 100% resepectivly) than without H.pylori infection (44.23 ± 7.44 years & 0% resepectively) (P= 0.001&0.024 respectivly). There were no statistical significant difference

between diabetic patients with and without H.pylori infection as regard the prevalence of female sex and durations of DM. (p=1.000 & 0.136 respectivly). Table (3)

As regard the mean duration of dyspepsia, it is statistically significantly higher in diabetic patient with H.pylori (6.47 ± 1.94weeks) than those without H.pylori infection (4.62 ± 1.98 weeks). (P= 0.022). As regard the prevalence of dyspeptic symptoms, the prevalence of diabetic neuropathy and the mean systolic and diastolic blood pressure, there were no statistical significant difference between diabetic patients with and without H.pylori infection (P>0.05).Table (4)

The mean fasting blood sugar (FBS), the prevalence of HBA1c > 7 , The prevalence of Microalbuminuria & the mean 24 hours protein were statistically significantly lower (171.62 ± 111.35 mg/dl, 38.5%, 30.8% & 65.54 ± 87.79mg/24h respectively) in H.pylori negative patients than in H pylori positive patients (240.59±101.38 mg/dl, 88.2% , 82.4% 191.35±112.06mg/24h respectively) (p=0.013,0.007, 0.004&0,002 respectively). Table (5)

There were no statistical significant difference between diabetic patients with and without H. pylori infection as regard the endoscopic findings. (P> 0.05).Table (6)

The prevalence of chronic active gastritis was statistically significantly higher in diabetic patients with than without H.pylori infection. (p=0.001). While the prevalence of chronic quiscent gastritis was statistically significantly lower in diabetic patients with than without H.pylori infection (p<0.001). There were no statistical significant difference between diabetic patients with and without H.pylori infection as regard the prevalence of gastritis with metaplasia. (P> 0.05).Table (6).

Table (1) The prevalence of H. pylori infection by CLO test and histopathological study in both groups

	Non Diabetic (n=30)		Diabetic (n=30)		χ^2p
	No.	%	No.	%	
CLO test					
Negative	10	33.3	16	53.3	0.118
Positive	20	66.7	14	46.7	
H. Pylori by histopathology					
Negative	9	30.0	13	43.3	0.284
Positive	21	70.0	17	56.7	

p: p value for comparing between the two studied groups

χ^2: Chi square test

*: Statistically significant at p ≤ 0.05

Table (2): H. Pylori positive cases by CLO test and histopathology in both groups

H. Pylori +ve cases	CLO test	H. Pylori by histopathology	χ^2 (p)
Non Diabetic	20	21	0.093 (0.761)
Diabetic	14	17	

p: p value for comparing between the two studied groups

χ^2: Chi square test *: Statistically significant at p ≤ 0.05

Table (3): Relation between H. pylori infection with demographic and clinical data in diabetic cases

	H. pylori by histopathology				Test of sig.
	Negative (n=13)		Positive (n=17)		
	No.	%	No.	%	
Sex					
Male	2	15.4	3	17.6	$^{FE}p=1.000$
Female	11	84.6	14	82.4	
Age					
Min. – Max.	30.0 – 55.0		40.0 – 70.0		
Mean ± SD	44.23 ± 7.44		58.88 ± 6.70		$^{t}p <0.001^{*}$
Median	44.0		60.0		
BMI (kg/m^2)					
<30	13	54.2	11	45.8	FEp=0.024
≥30	0	0.0	6	100	
Duration of DM (years)					
Min. – Max	5.0 – 17.0		4.0 – 22.0		MWp=0.136
Mean ± SD.	10.23 ± 4.09		12.76 ± 4.75		
Median	8.0		13.0		

p: p value for comparing between H. pylori categories FE: Fisher Exact test
t: Student t-test MW: Mann Whitney test χ^2: Chi square test

Table (4): Relation between H. pylori with medical history in diabetic cases

	H. pylori by histopathology				Test of sig.
	Negative (n=13)		Positive (n=17)		
	No.	%	No.	%	
Duration of dyspepsia (weeks)					
Min. – Max.	2.0 – 8.0		3.0 – 9.0		$^{MW}p=0.022^{*}$
Mean ± SD.	4.62 ± 1.98		6.47 ± 1.94		
Median	4.0		7.0		
Vomiting	5	38.5	6	53.3	$^{FE}p=1.000$
Heart burn	9	69.2	10	58.8	$^{FE}p=0.708$
Abdominal pain	11	84.6	14	82.4	$^{FE}p=1.000$
Peripheral Neuropathy	9	69.2	10	58.8	$^{FE}p=0.708$
Systolic blood pressure					
Min. – Max.	90.0 – 130.0		90.0 – 130.0		
Mean ± SD.	116.15 ± 14.46		107.06 ± 13.12		0.082
Median	120.0		100.0		
Diastolic blood pressure					
Min. – Max.	60.0 – 90.0		60.0 – 90.0		
Mean ± SD.	76.15 ± 9.61		71.18 ± 7.81		0.129
Median	80.0		70.0		

p: p value for comparing between H. pylori categories FE: Fisher Exact test
MW: Mann Whitney test *: Statistically significant at p ≤ 0.05

Table (5): Relation between H. pylori infection with FBS, HBAIc and Microalbuminuria in diabetic patients

	H. pylori by histopathology		Test of sig.
	Negative (n=13)	Positive (n=17)	
FBG(mg/dl)			
Min. – Max.	110.0 – 526.0	105.0 – 526.0	
Mean ± SD.	171.62 ± 111.35	240.59 ± 101.38	$^{MW}p= 0.013^{*}$
Median	140.0	225.0	
HBA1c			
6.5 – 7	8 (61.50%)	2 (11.8%)	$^{FE}p= 0.007^{*}$
>7	5 (38.5%)	15 (88.2%)	
Min. – Max.	6.10 – 12.50	8.20 – 11.40	
Mean ± SD.	7.91 ± 2.41	9.46 ± 0.91	$^{t}p= 0.044^{*}$
Median	6.40	9.30	
Microalbuminuria	4 (30.8%)	14 (82.4%)	$\chi^2p= 0.004^{*}$
Urine protein 24 hour (mg/24h)			
Min. – Max	13.0 – 275.0	14.0 – 300.0	
Mean ± SD.	65.54 ± 87.79	191.35±112.06	$^{MW}p=0.002^{*}$
Median	16.0	230.0	

p: p value for comparing between H. pylori categories FE: Fisher Exact test t: Student t-test
MW: Mann Whitney test χ^2: Chi square test *: Statistically significant at p ≤ 0.05

Table (6): Relation between H. pylori endoscopic finding in diabetic cases

	H. pylori by histopathology				Test of sig.
	Negative (n=13)		Positive (n=17)		
	No.	%	No.	%	
Gastritis	13	100.0	17	100.0	-
Duodenitis	2	15.4	5	29.4	0.427
Hiatal hernia	0	0.0	1	5.9	1.000
GERD	0	0.0	3	17.6	0.238
Erosion	1	7.7	4	23.5	0.355
Mass	0	0.0	0	0.0	-

Table (6): Relation between H. Pylori with histopathological findings in diabetic

	H. Pylori				FEp
	Negative (n=13)		Positive (n=17)		
	No.	%	No.	%	
Chronic active gastritis	4	30.8	16	94.1	0.001^{*}
Chronic quiscent gastritis	9	69.2	1	5.9	$<0.001^{*}$
Gastritis with metaplasia	1	7.7	1	5.9	1.000

FE: Fisher Exact test
*: Statistically significant at p ≤ 0.05

Discussion:

The link between diabetes and HP infection has been inconsistently reported. Researchers have hypothesized an association between infection with Helicobacter pylori and diabetes mellitus.[31&32] However, studies to date have failed to confirm this hypothesis as results have been discordant.[33-35] The aim of this study was to determine the prevalence of Helicobacter pylori infection in type 2 diabetes mellitus patients with non ulcerative dyspepsia and to evaluate the relationship between H. pylori infection and the glycemic control and microalbuminuria. We studied two groups of patients, group I included 30 patients non diabetic with dyspepsia and group II included 30 patients diabetic with dyspeptic symptoms.

In our work, assessment of Helicobacter pylori (HP) Infection was done by both rapid urease test, also known as the Campylobacter-like organism (CLO) test and histological study of antral and corporal biopsies. When either the histopathology or the CLO tests were positive, the infection was confirmed.[36] The prevalence of HP infection was insignificantly higher in histopathological results than in CLO test .Since the diagnosis of HP has a sensitivity of 93–99% and specificity of 95-99%, the diagnosis established by the histopathological method accepted as the "gold standard".[36]

The relationship between DM and HP infection is controversial. In our study we found that the prevalence of H pylori infection by both CLO test and histopathological study was statistically insignificantly lower in diabetic than in non diabetic.

In agreement with our work, Demir et al[37] and other investigator,[38-40] showed that the prevalence of HP infection did not differ significantly between diabetic patients and nondiabetic controls.

The decreased chance of growth of HP in diabetics may due to presence of microangiopathy of gastric mucosa which may decrease the mucous layer which is essential for H pylori survival. [41]

On the contrary Bener A, et al [31] and other investigator [42] reported that Helicobacter pylori infection was significantly higher in diabetic patients than non-diabetic subjects. Several hypotheses were presented for confirmation of higher prevalence of HP infection in diabetic patients such as immune system impairment (cellular and humoral immunity) in patients with diabetes mellitus, the reduction of both gastrointestinal motility and acid secretion (autonomic neuropathy) and higher secretion of pro-inflammatory cytokines related to the HP gastric infection itself.[31&42]

In our work we found that significantly higher mean age was associated with HP infection in diabetic patients.

Pounder RE, Ng D.[43] stated that there is positive correlation between HP infection and increasing age of the subjects. Also MalatyHM et al[44] stated that the risk of HP infection increases with advancing age. These associatiation could be explained by repeated exposure to the infection in old subjects.

In our work we found that significantly higher BMI were associated with HP infection in diabetic patients.

Also Cho I, Blaser MJ, Francois F, et al.[45] concluded that, among older individuals and especially those with a higher BMI, glucose intolerance associated with HP could remain significant.

Glycated hemoglobin (HbA1c) results from the non enzymatic glycosylation of hemoglobin, reflecting integrated blood glucose levels during the preceding 3–4 months.[46-48] HbA1c levels are predictive of both prevalent and incident diabetes and are useful in diagnosing prediabetes, diabetes and long term control of diabetes.[46-48]

In our study we found that diabetic patients with HP infection were characterized by poor diabetic control as regard significantly higher mean fasting blood sugar and high prevalence of HAIC > 7 than in HP negative patients.

In agreement, Buell C et al [46] found a positive association between HP status and HbA1c levels. Also, Cho I and Blaser[45] report that HP seropositivity, and especially HP cagA positivity, was associated with higher mean

HbA1c levels, an association that persisted after excluding individuals with a history of diabetes mellitus and controlling for potential confounders.

The most plausible hypothesis is that H. pylori directly or indirectly increases levels of HbA1c H. pylori plays a role in the regulation of leptin and ghrelin, [49&50] which are central to energy homeostasis and metabolism.[51] These 2 hormones are involved in the regulation of appetite and energy expenditure. Ghrelin decreases energy expenditure and promotes weight gain,[52] whereas leptin, which is expressed mainly by adipocytes, reduces food intake and increases energy expenditure. [53]HP infection was associated with lower levels of circulating ghrelin through decreases in the ghrelin producing cells in the gastric mucosa and increases in gastric leptin levels.[54]

Gino et al[55] also found a poor glycemic control in type two DM patients who were infected by HP. The significant decrease in the glycemic control in patients infected with the virulent CagA-positive of HP could be explained by its ability to increase insulin resistance,[56] and decrease serum concentration of somatostatin,[57] which has an inhibiting effect on insulin release.[57] Also, CagA-positive strains are associated with increased production of cytokines such as tumor necrosis factor (TNF alpha), interleukin (IL)-1, -6, and -8,2 which may affect carbohydrate metabolism and stimulate the secretion of insulin counter-regulatory hormones leading to hyperglycemia in diabetic patients.[58]

In contrast, other studies did not find an association between infection by HP and glycemic control,[59] and explained their findings by the fact that gastritis increases glucose-and meal-stimulated insulin release by increasing gastrin secretion, which inhibit glucose absorption in the small intestine, and amplifies glucose stimulated insulin release.[59]

Obesity is an established risk factor for diabetes and it is known that high BMI is associated with elevated HbA1c. Separately, the presence of HP is also associated with elevated HbA1c. In the present study we hypothesized that having both high BMI and the presence ofHP would have a synergistic effect, increasing HbA1c even more than the sum of the individual effect of either risk factor alone.

In agreement, Yu Chen and Martin Blaser[60] showed a synergistic effect of H. pylori and body mass index (BMI) on increased levels of HbA1c in that higher levels were found in HP –infected subjects with BMI >25.

Microalbuminuria (Malb) is a confirmed marker of diabetic nephropathy.[61] The appearance of albumin in urine is thought to be the consequence of generalized endothelial damage along the vascular area including the glomerulus.[61] Various infectious diseases may be listed among the etiologic factors related with this vascular endothelial damage and consequently developing atherosclerosis. As shown in recent studies, HP are these microorganisms.[62-65]

In our study microalbuminuria was present in 4 out of 13(30.8%) of H pylori negative patients and present in 14 out of 17 (82.4%) of H pylori positive patients. This relation was found to be statistically significant. Also the mean 24 hours protinuria was statistically significantly higher in diabetic with than without HP.

In agreement, Pietroiusti A, et al[66] showed that virulent strains of HP are associated with microalbuminuria in type 2 diabetic patients. This may be due to an immune-mediated injury at the level of the endothelium caused by a systemic immune response to the infection, leading to albumin leakage.[67&68] Although a certain value has not been determined about the frequency, it has been demonstrated that persistent systemic inflammatory response related with HP increases the vascular injury in diabetics and predisposes them to pulmonary, cardiovascular and cerebral diseases.

HP infection has been hypothesized to contribute to a strong inflammatory response, atherogenesis and plaque instability.[69] It is thought that pro-inflammatory factors are produced at excessive amounts in this infection, and cross-reaction between the released mediators and host antigens causes

gastric injury and extra-digestive manifestations. Studies have demonstrated a significant relation between LPa, HDL-C, oxidant lipids, LDL-C, thrombotic activation-related anti-thrombin (AT)-III, von-Willebrand factor, interleukin-1, tumor necrosis factor, and interleukin-6 and HP infection.[67&68]

Oshima et al[65] reported that HP was associated with elevated C-reactive protein and soluble intercellular adhesion molecule-1. This indicates that chronic HP infection might be involved in the pathogenesis of atherosclerosis which is the corner stone of the development of microalbuminuria.

Conclusion: No statistical significant difference in the prevalence of H.pylori between diabetic and non diabetic control H.pylori infection was significantly associated with poor glycemic control and high prevalence of microalbuminuria in diabetic patients.

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Original Article

Relationship Between Migraine, Calcitonin Gene Related Peptide and Obesity in Females During The Reproductive Period and Menopause

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Abstract:

Objective: It is now generally accepted that the neuropeptide calcitonin gene related peptide (CGRP) plays a significant role in migraine pathogenesis. The aim of the present work is to investigate the relation between obesity, CGRP and migraine in women of both the fertile and menopausal age groups. **Methods:** The study is a prospective study performed in El-Hadara Hospital, Alexandria University. The study was conducted on 80 women, who were subdivided into Group I, including 40 fertile women and group II, including 40 menopausal women. Each group was further subdivided into two subgroups: group Ia, IIa included 20 migraineures each and group Ib, IIb included 20 healthy females each, serving as controls. All migraineures were selected from Neurology Outpatient Clinic. Measurement of body mass index (BMI) and waist/hip ratio (WHR), estimation of serum CGRP, adiponectin and estradiol were done to all participants. The migraine Disability Assessment Score (MIDAS) was applied to all migraineurs. **Results:** Migraineurs had a higher serum CGRP than controls in both fertile and menopausal groups. There was an increase in CGRP level and frequency of attacks of migraine in obese patients compared to non obese patients, as well as in patients with visceral obesity compared to those with normal WHR regardless of their BMI. Serum adiponectin level was observed to be significantly reduced in migraineurs (fertile & menopausal) than controls. Moreover, no significant difference was detected between fertile migraineurs and menopausal migraineurs regarding serum adiponectin level. Also our study revealed that the obese migraineurs had a significant lower level of adiponectin than non-obese.In addition, the serum adiponectin level was significantly reduced in migraineurs with visceral obesity than in migraineurs without visceral obesity **Conclusion:** Female sex steroids are not the only factor for inducing migraine. Obesity and visceral obesity (even with normal BMI) may be an important link between CGRP and migraine that may offer an explanation to the occurrence of migraine in menopausal women with low estrogen.

Keywords: migraine, calcitonin gene related peptide, obesity, visceral obesity reproductive period, menopause

Introduction:

Migraine is a common neurological disorder characterized by recurrent episodes of headache that is about two to three times more common in women than in men[1]. Although much debate has occurred around the exact mechanism of migraine, it is now generally accepted that the neuropeptide calcitonin gene related peptide (CGRP) plays a significant role in migraine pathogenesis[2]. CGRP is a 37-amino acid neuropeptide that belongs to the calcitonin family of neuropeptides and promotes a wide range of biological effects including neuromodulation of nociceptive input, promotion of neurogenic inflammatory pain, and vasodilatation[3,4]. CGRP is expressed in many tissues including the dorsal root ganglia, which contain the cell bodies of the sensory nerves that terminate peripherally on blood vessels, as well as in the perivascular sensory nerve terminals, which have been shown to be a major source of circulating CGRP[5]. CGRP released from trigeminal neurons under the effect of various triggers causes dilatation of dural vessels and facilitation of pain transmission inducing migraine attacks[2,6]. The exact triggers causing the increased release or increased sensitivity to CGRP or both, in migrainuers is not yet completely elucidated. CGRP is largely influenced by sex steroids, and female sex

hormones have been implicated in pathogenesis of migraine[7]. Data indicate that changes in levels of female hormones like17 β-estradiol may be involved in the pathogenesis of migraine. Some reports suggest that migraine attacks are precipitated by a background of increased estrogen as that occurring during pregnancy,[8,9] while others suggest that estrogen withdrawal as that occurring during menstruation[10] and perimenopausal period,[11] is a trigger for migraine. Hence, the female sex steroids can either trigger or suppress migraine attacks, or may be that estrogen is not the only underlying factor that influences CGRP release and migraine attacks. Previous studies have found a link between migraine and obesity as estimated by anthropometric measurements[12]. Bigal et al[13] found that the frequency and severity of migraine attacks increased in patients having a body mass index greater than thirty. In addition patients with visceral obesity were found to have a high level of inflammatory markers and CGRP, suggesting that visceral obesity could be another risk for migraine[14]. However the relation between obesity, especially visceral fat accumulation, and migraine in both fertile and menopausal women has not been fully elucidated.

Aim:

The aim of the present study was to investigate the relation between obesity, CGRP and migraine in women of both fertile and menopausal age groups.

Subjects and Methods:

This study is a prospective study performed in El-Hadara Hospital, Alexandria University. The study was conducted on 80 females. classified into 2 groups:

Group I: included 40 fertile women, who ranged from 25-40 years of age, with a mean 33.05 years. They were further subdivided into: group Ia, including 20 fertile migraineures and group Ib, including 20 fertile healthy females as controls.

Group II**:** included 40 postmenopausal women, who ranged from 52-65 years of age, with a mean 57.2 years. They were further subdivided into: group IIa, including 20 postmenopausal migraineures and group IIb, including postmenopausal healthy females as controls.

Migraineures were selected from the Neurology Outpatient Clinic, Main University hospital after an informed consent was taken according to the declaration of Helsinki approved by the committee of Medical ethics, Alexandria faculty of Medicine. All patients fulfilled the international headache society (IHS) diagnostic criteria for migraine[15]. Patients were asked to stop medication 3 days before the study and no vasoconstrictor or dilators drugs were taken before examination. Full history taking, physical and neurological examinations were performed to exclude headache secondary to organic or systemic metabolic disease.

All participants were subjected to the following: 1- Full history taking with special attention to headache. 2- Full neurological and physical examination. 3- Measurement of body mass index (BMI) and waist/hip ratio (WHR). 4-Estimation of serum level of CGRP: serum CGRP was measured by using human CGRP enzyme immunoassay (EIA) Kit manufactured by Cayman Chemical Company, USA. This enzyme-linked immune sorbent assay (ELISA) is based on a double-antibody sandwich technique that permits measurement of CGRP within the range of 0-1,000 pg/ml, with a detection limit of <5 pg/ml[16]. 5-Estimation of serum level of adiponectin: Serum adiponectin was measured by using Quantikine DRP 300 ELISA kit. Manufactured by R&D systems Inc. Minneapolis, USA[17]; 6- Estimation of serum estradiol level[18].

All the migraineures were examined in between the attack of migraine (days without any type of headache for at least 72 hours prior to their examination). The Migraine Disability Assessment Score (MIDAS)[19,20] was applied to all patients. Patients were asked questions about the frequency and duration of their headaches as well as how often these headaches limited their ability to participate in activities at work, or at home. The scores were as follows: patients with little or no disability or, MIDAS Grade I were given a score from 0 to 5; Patients with Mild disability or MIDAS Grade II were given a score from 6 to 10; patients with Moderate disability or MIDAS Grade III were given a score from 11 to 20; and patient with

severe disability, or MIDAS Grade IV were given a score of 21 or above.

Statistical analysis:

After data entry into a specially designed sheet using Microsoft Excel, a print out of the data was thoroughly revised and data entry mistakes were corrected. Then the file was transferred into Statistical Package for Social Science (SPSS) version 17 format. Normality was tested using KS test and data were proved to be normally distributed (p>0.05), so mean and standard deviation were used for descriptive statistics and parametric tests were used for comparison. Frequency of attacks and MIDAS were reported using Median and inter-quartile range (IQR). The following statistical tests were carried out:

Descriptive statistics: minimum, maximum, mean and standard deviation, median and Inter-quartile range, comparison of two independent means using Student's t-test, comparison of more than two means using Analysis of variance (Anova) test, post-hoc was carried out using Scheffe method. For categorical data, cross tabulation and Chi square testing were carried out. Box and Whisker graph was used for presentation of continuous (scale) variables. Pearson's correlation was also performed.

Results:

The present study revealed that mean serum CGRP was significantly higher in migraineurs than in controls in both fertile and menopausal women. Moreover, the fertile migraineurs had a significantly higher mean serum CGRP than menopausal migraineurs (F= 63.769, P<0.001) (Fig 1, table I)

On classifying migraineurs into obese and non- obese according to BMI (obesity was defined as BMI≥30). It was found that obese migraineurs had a significant higher mean serum CGRP than non-obese (t= 6.542, P<0.001) (table II). In addition migraineurs with visceral obesity (WHR≥0.8 were found to have a significantly higher mean serum CGRP than migraineurs without visceral obesity (WHR< 0.8) (t= 3.305, P=0.002) regardless of BMI (table III).

In fertile and menopausal migraineurs, a positive correlation was detected between serum CGRP and BMI (r=0.925, P<0.001 for fertile group & r =0.925, P<0.001 for menopausal group); as well as with WHR (r=0.872, P<0.001 for fertile group & r=0.882, P<0.001 for menopausal group). A positive correlation was also found between serum CGRP and serum estradiol level in fertile migraineurs (r= 0.862, P<0.001), but no significant correlation was detected in menopausal migraineurs (r=0.150 & p=0.527). However, no significant correlation was noticed between CGRP and BMI, WHR, estradiol in fertile control group (r=0.150, P=0.527 for BMI, r=0.123, p=0.606 for WHR, r= 0.132, P=0.578 for estrogen) or in menopausal control group (r=0.168, p=0.478 for BMI, r=0.127, p=0.592 for WHR, r=0.329, p=0.157 for estradiol).

The frequency of attacks of migraine were significantly higher in the fertile group than in the menopausal group (Z =3.646, P=0.001). 50% of fertile migraineurs had 6 attacks/ month, 30% had 5 attacks/ month and 20% had only 4 attacks / month. On the other hand, 60% of menopausal migraineurs had 4 attacks/ month and 40% had 5 attacks/ month.

A significant positive correlation was observed between serum estradiol level and frequency of attacks in fertile migraineurs (r=0.619 & p=0.004). A similar positive correlation was detected between frequency of attacks and serum CGRP, BMI and WHR (r=0.707& P<0.001 for CGRP, r=0.731, P<0.001 for BMI and r=0.791 and P<0.001 for WHR).

On the other hand, the menopausal migraineurs showed no significant correlation between frequency of attacks and serum estradiol level (r=0.123 & P= 0.606), whereas a positive correlation was found between frequency of attacks and CGRP (r=0.617 & p=0.004), BMI (r=0.750& P<0.001) and WHR (r= 0.715& P<0.001)

Moreover, the obese migraineurs had higher frequency of attacks than the non-obese, where 44.4% of the obese had 6 attacks/ month compared to only 9.1% for the non-obese. Also 44.4% of obese and only 27.3% of non-obese had 5 attacks. On the reverse the majority of non–obese (63.6%) had 4 attacks /month whereas only 11.2% of obese had the same number of attacks.

When comparing frequency of attacks in migraineurs with visceral obesity and migraineurs without visceral obesity, 33.3% of migraineurs with visceral obesity had 6 attacks /month, 50% had 5 attacks and 16.7%

had 4 attacks. On the other hand, only 20% of migraineurs without visceral obesity had 5 attacks and 80% had 4 attacks and no had 6 attacks / month.

On applying the migraine Disability Assessment Score (MIDAS) to all migraineurs, the present study revealed that 18 migraineurs were classified as grade I, while the other 22 migraineurs were grade II. On comparing mean CGRP levels in grade I &II migraineurs, it was found that grade II migraineurs had significantly higher level (55.2+7.1 pg/ml) than grade I (45.7+5.5 pg/ml) (t=4.59 & P<0.001)

In addition, the present study showed that most of obese migraineurs were grade II (94.4%), whereas most of non-obese were grade I (77.3%). This finding was also detected when relating MIDAS to visceral obesity where 66.7% of migraineurs with visceral obesity were classified as grade II, whereas 80% of migraineurs without visceral obesity were grade I.

In the present study, the serum adiponectin level was observed to be significantly reduced in migraineurs (fertile & menopausal) than controls. Moreover, no significant difference was detected between fertile migraineurs and menopausal migraineurs regarding serum adiponectin level. (F= 19.59 & P<0.001) (table I). Also our study revealed that the obese migraineurs had a significant lower level of adiponectin than non-obese migraineurs (t=8.507 & P<0.001) (table II). In addition, the serum adiponectin level was significantly reduced in migraineurs with visceral obesity than in migraineurs without visceral obesity (t= 2.828 & P=0.007) (table III).

Table I: The anthropometric measurements, mean serum CGRP, estradiol and adiponectin levels in all studied groups:

characteristic	Group Ia (n=20)	Group Ib (n=20)	Group IIa (n=20)	Group IIb (n=20)	Test of significance
Serum CGRP (pg/ml)	54.4 ± 8.6 [a]	37.1 ± 4.9 [b]	47.5 ± 5.6 [c]	30.04±4.00 [d]	F=63.769* P<0.001
BMI (Kg/m2)	28.5 ± 3.1 [a]	27.1 ± 2.3 [a]	30.3 ± 2.7 [b]	29.2 ± 2.2 [b]	F=4.883* p<0.01
WHR	0.88±0.09 [a]	0.87±0.06 [a]	0.89± 0.08 [a]	0.91 ± 0.07 [a]	F=0.957 p=0.418
Serum Estradiol (pg/ml)	68.9 ±12.6 [a]	62.6±9.5 [a]	25.7±1.8 [b]	22.1 ± 2.3 [b]	F=182.005* P<0.001
Serum Adiponectin (µg/ml)	12.4±5.1 [a]	21.1±5.5 [b]	12.2±4.7 [a]	20.6 ± 4.4 [b]	F=19.594* P<0.001

Group Ia: fertile migraineure **Group Ib:** fertile controls **Group IIa:** postmenopausal migraineures **Group IIb:** postmenopausal controls
Data are mean ± SD *Significant at p level < 0.05.
The same small letter has no significant differences. The different small letters have significant differences.

Table II: Mean serum CGRP (pg/ml), adiponectin (µg/ml) and estradiol (pg/ml) levels in obese and non-obese migraineurs:

	Obese Migraineurs (n= 18)	Non-obese migraineurs (n= 22)	Test of significance
CGRP (pg/ml)	57.3± 5.8	45.7±5.3	t=6.542* P<0.001
Adiponectin (µg/ml)	8.005±0.43	15.9±3.9	t=9.404* P<0.001
Estradiol (pg/ml)	53.17±26.9	42.64±19.9	T=1.377 P=0.178

Data are mean ± SD *Significant at p level < 0.05.

Table III: Mean serum CGRP (pg/ml), adiponectin (µg/ml) and estradiol (pg/ml) levels in Migraineurs with and without visceral obesity

	Migraineurs with visceral obesity (n= 30)	**Migraineurs without visceral obesity (n= 10)**	**Test of significance**
CGRP (pg/ml)	53.15± 7.6	44.52±5.1	t=3.305* P<0.01
Adiponectin (µg/ml)	11.18±4.8	15.8±3.3	T= 2.828* P<0.01
Estradiol(pg/ml)	48.66±25.2	43.53±18.7	t=0.685 P=0.501

Data are mean ± SD *Significant at p level < 0.05.

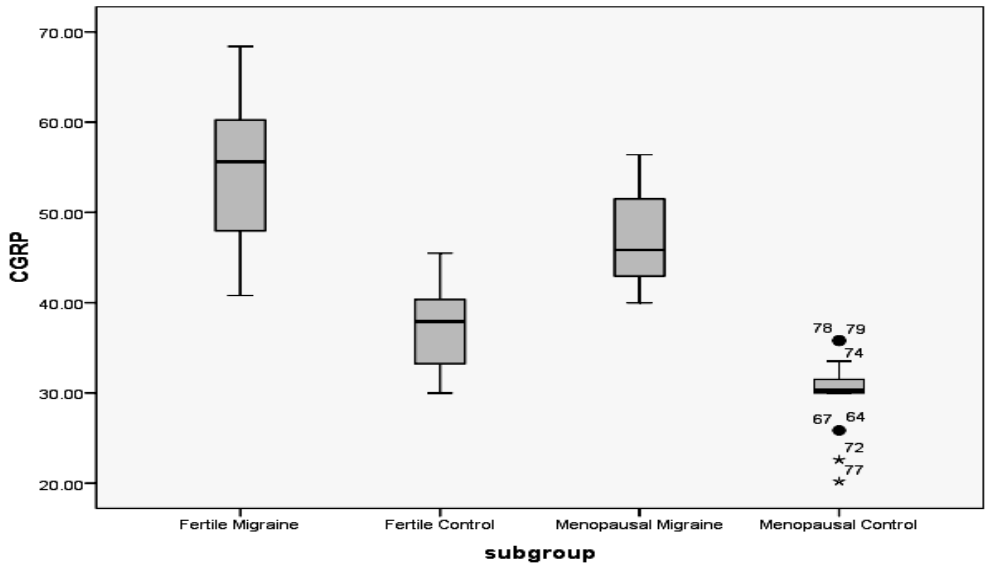

Figure (1): mean serum CGRP in fertile migraineurs, fertile controls, menopausal migraineurs and menopausal controls

Discussion:

The results of the present study showed that migraineurs had a higher serum CGRP than controls in both fertile and menopausal groups as measured in between the attacks. CGRP was also found to be significantly higher in patients classified as grade II according to MIDAS than grade I patients. These results are in accordance with the results of Ashina et al.[21] who demonstrated that CGRP is increased in cubital venous blood of migraineurs outside of attack. Several studies have provided evidence for the involvement of CGRP in migraine pathogenesis. First a report provided evidence for increased CGRP levels in blood of the external jugular vein during the attack in migraine patients[22] In addition another study demonstrated that intravenous administration of alpha-CGRP was able to trigger headache in migraine patients[23]. Other studies also demonstrated the efficiency of the use of CGRP antagonists in migraine treatment[24,25]. CGRP receptors are located on several sites, It has been proposed that, after release of CGRP from the nerve endings of primary sensory neurons during a migraine, it activates CGRP receptors. Activation of receptors located around meningeal vessels, causes vasodilation,[26] while activation of those located on mast cell causes release of cytokines, inflammatory mediators and plasma extravasation.[27] It has also been shown that migraineurs may not only exhibit increased level of CGRP, but increased sensitivity to CGRP actions as well, which has

been attributed to an elevated receptor activity-modifying protein-1 (RAMP1), a subunit of the CGRP receptor [28].

The present study showed that the frequency of migraine attacks was higher in fertile migraineurs than menopausal migraneurs and that fertile migraineurs had a higher CGRP level than menopausal migraineurs. These results suggested that female sex steroids seem to be involved in the pathogenesis of migraine and are supported by the detection of a positive correlation between serum estradiol level & frequency of migraine in fertile migraineurs, but not in menopausal migraineurs. It therefor appears that presence of higher estrogen level in fertile migrainuers may be an underlying cause for the occurrence of migraine. The predominant effect of estrogen appears to be facilitation of the glutamatergic and serotonergic systems. In addition, it has both facilitatory and inhibitory effects on the opiatergic, GABAergic and noradrenergic systems which may induce modulation of CGRP[8]. However the exact relationship between CGRP and estrogen is not completely elucidated, most data point to estrogen withdrawal as that occurring just before menstruation and during perimenopause as being a trigger to the onset of migraine[1]. On the other hand, migraine attacks have been known to occur in other phases of the menstrual cycle, when estrogen level is higher and have been characterized as non menstrual migraine,[29] in addition migraine has been also known to occur after menopause when there is a steady low estrogen level[30]. This discrepancy has led to the notion that estrogen may not be the only player affecting CGRP levels in migraine and that CGRP may be increased due to other factors.

On classifying migraineures as obese (BMI ≥30) & non obese (BMI ≤ 30), the frequency of attacks of migraine were higher in obese patients than non obese patients, although no significant difference in estradiol level was detected between the two groups. These results suggested that estrogen is not the only factor for inducing migraine. Moreover, a positive correlation was also detected between frequency of migraine attacks and BMI. In addition, most of the obese patients were classified as grade II (MIDAS). This suggested that obesity may contribute in some way by augmenting the mechanisms involved in migraine attacks. There has been recent data suggesting that obesity and migraine are associated in several ways. Obesity is a state of low grade systemic inflammation as adipose tissue secretes pro-inflammatory cytokines and adipocytokines that have been implicated in migraine.[31] Obesity may also induce sympathetic activation, which can contribute to the increase in headache frequency[32].

On comparing serum CGRP level in obese & non-obese migraineures there was an increase in CGRP in the obese subgroup than the non obese subgroup. The association between obesity and CGRP has been suggested by Recober et al[33] who proposed that in obese susceptible individuals there is increased trigeminal CGRP production which may facilitate the occurrence of migraine attacks.

Moreover, the present study revealed that the migraineurs with visceral obesity as diagnosed by increased WHR (≥ 0.8) also had higher CGRP levels than those with normal WHR (≤ 0.8) regardless of their BMI, and also had higher frequency of attacks than migraineurs with normal WHR. In addition, most of patients with visceral obesity were classified grade II according to MIDAS. These findings suggested that regional obesity may also play a role in the pathogenesis of migraine, and are supported by a study that demonstrated the expression of CGRP in abdominal fat, and the increased CGRP mRNA levels in abdominal subcutaneous fat in postmenopausal women compared with premenopausal women,[34] highlighting the presence of high levels of CGRP in postmenopausal women even though they had low estrogen level.

There is evidence demonstrating an association between migraine attacks and inflammatory mediators, as adiponectin, tumor necrosis factor alpha (TNF-alpha), and interlueken 6, (IL-6) [35].TNF-alpha has been found to increase activity and secretion of CGRP in rat trigeminal ganglion neurons,[36] while adiponectin has been shown to be protective against migraine through its anti-inflammatory properties[37]. In the present study serum adiponectin level was observed to be significantly reduced in migraineurs (fertile & menopausal) than controls. Moreover, adipnectin levels were found to be significantly lower in obese migraineurs than non-obese, and in migraineurs with high visceral obesity

than those with normal WHR, although some migraineurs with visceral obesity had normal BMI (12 patients out of 30). These finding suggested that adiponectin may be a player affecting the relation between pathogenesis of migraine and obesity. Adiponectin functions to decrease the levels of TNF-alpha, reduce C reactive protein[38] and has been shown to decrease in cases of obesity[39]. When deficient in obesity the resultant increase of inflammatory mediators can elevate circulating levels of CGRP, or increase sensitivity to otherwise normal levels of CGRP, and may decrease the threshold for precipitating migraine attacks leading to increased frequency of migraine attacks by making the patient more susceptible to their usual triggers.

In conclusion, although female sex steroids seem to be involved in the pathogenesis of migraine by affecting CGRP level especially in fertile women, they are not the only factor for inducing migraine. Obesity and regional obesity (even with normal BMI) are another factor associated with migraine and may be an important link between CGRP and migraine that may offer an explanation to the occurrence of migration in menopausal women with low estrogen. The significantly lower level of adiponectin found in obese migraineurs and in those with visceral obesity may explain the increase activity and secretion of CGRP in such patients.

Recommendations:

Further studies are needed to examine the effect of weight reduction and loss of visceral obesity in the frequency of migraine attacks and level of CGRP and adiponectin. This will result in the identification of novel therapeutic targets that will have a great impact in patients and in our society.

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Original Article

Effect of Bariatric Surgery on Serum Glucagon like Peptide-1 Concentration and Metabolic Parameters in Obese Type 2 Diabetics.

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Abstract:

Background: Bariatric surgery has proven an effective anti-diabetic treatment modality. It results in significant weight loss and improvement in health and quality of life. Roux-en-Y gastric bypass (RYGB) surgery has been shown to be associated with lower overall mortality for subjects with T2DM. **Objective:** Assess the effect of RYGB surgery on serum glucagon like peptide-1 (GLP-1) concentration, metabolic and biochemical parameters pre and postoperatively in type 2 diabetics. **Methods:** This prospective study comprised 50 subjects divided into 3 groups. **Group I:** 20 obese diabetics (BMI > 35) that underwent Roux-en-y gastric bypass surgery (RYGB); **group II:** 20 lean subjects with T2DM and **group III:** 10 lean subjects as healthy controls. BMI, fasting blood glucose and 2hPP glucose, HbA1c, lipid profile, fasting serum insulin, fasting and 2hP plasma GLP-1were estimated. In group 1, tests were done pre and three months postoperatively. Insulin resistance was quantified using HOMA-IR. **Results:** Fasting and 2hPP GLP-1 levels were lower in group 1 compared to the other 2 groups respectively (FGLP-1: 4.33±1.10vs. 5.21±0.89, 6.14±0.42 ng/ml, p<0.001), 2hPP GLP-1 (4.94 ± 1.17 vs. 6.04 ± 0.99, and 9.32±0.97ng/ml, p<0.001); also the difference between groups 2 and 3 was statistically significant. Fasting and 2hPP GLP-1 levels showed significant negative correlation with all metabolic parameters except HDL-C, which showed a positive correlation (p<0.001). Regarding the postoperative results, we found an increase in the fasting GLP-1 levels (4.53 ±1.02vs 4.3±1.1 ng/ml, *p*=0.09), 2hPP GLP-1 levels (9.79±1.99 vs 4.9 ± 1.2 ng/ml, p<0.001) and HDL-C. Also, there was a statistically significant decrease in all metabolic parameters. Sixty percent of the patients stopped their anti-diabetic medications postoperatively. **Conclusion:** RYGB surgery resulted in a statistically significant reduction in BMI and all metabolic parameters. Fasting & 2hPP GLP-1 levels were low in diabetics. Postoperative 2hPP GLP-1 levels increased, possibly responsible for the metabolic benefits.

Keywords: Obesity, Type 2 Diabetes Mellitus (T2DM), glucagon like peptide-1 (GLP-1), HOMA-IR, Roux-en-y gastric bypass surgery (RYGB).

Introduction:

More people are diagnosed with type 2 diabetes mellitus due to increase in prevalence of obesity across continents. Conventional therapies seem to be unable to stop the progression of T2DM and certainly do not cure the disease[(1)]. In contrast, bariatric surgery has proven an effective anti-diabetic treatment modality. Additionally, it results in significant weight loss and improvement in health and quality of life[(2)]. Also, RYGB has been shown to be associated with lower overall mortality for subjects with T2DM. Currently the most commonly used form of bariatric surgery is RYGB[(3)].

Remission of T2DM and marked reduction of insulin resistance has been shown to occur a few days following RYGB operation, before any significant weight loss has taken place, indicating that the operation itself brings about acute endocrine changes improving glucose homeostasis[(4)].

Although the precise mechanisms through which type 2 diabetes remission occurs following metabolic surgery remain to be fully elucidated, it is clear that endocrine changes due to rearrangement of the gut

anatomy play an important role. Of the potential hormones involved in the endocrine changes are the in cretin hormones. Several studies have examined changes in levels of incretin hormones following bariatric surgery[5]. Stimulated GIP levels after a test meal were reported by Laferrère and colleagues to be increased 1 month after gastric bypass[6]. Several groups have reported decreased ghrelin levels after RYGB, which may partly account for the improved glycemia[7]. Also elevated PYY levels after gastric bypass have been reported by Valderas et al[8]. Early studies reported an increased fasting and postprandial enteroglucagon (previously used as a marker for GLP-1) after both gastric bypass and jejunoileal bypass[7]. Subsequent changes in both fasting and postprandial GLP-1 levels have been reported in several studies[5]. GLP-1 is known to stimulate insulin secretion from pancreatic *β* cells; inhibit glucagon secretion from pancreatic *α* cells and decrease gastrointestinal motility, appetite, and food intake and body weight[3].

Our aim was to assess the effect of Roux-en-Y gastric bypass surgery (RYGB) on serum glucagon like peptide-1(GLP-1) concentration and changes in metabolic and biochemical parameters pre and postoperatively.

Research design and methods:

Study Subjects:

The current prospective case-control study was conducted from April 2012 to December 2012 and this study was approved by the internal review board of Ain Shams University. All subjects provided written informed consent to undergo various examinations, and provide blood samples.

Our study was conducted on 50 subjects divided into 3 groups. **Group I:** 20 obese subjects with T2DM (BMI > 35) reviewed before and after Roux-en-y gastric bypass surgery; **Group II:** 20 lean subjects with T2DM (BMI<25 kg/m²) and **Group III:** 10 lean subjects as healthy controls. All patients in group 1 were on medical treatment, 9 of them were on insulin plus oral anti-diabetics while the remaining 11 were on oral anti-diabetics alone. All patients in group 2 were on medical treatment, 7 were on insulin plus oral anti-diabetics while the remaining 13 were on oral anti-diabetics alone. In group1, tests were done preoperative and three months after surgery.

All patients were recruited from the outpatient clinic of both the endocrinology and the bariatric and metabolic surgery departments of Ain Shams University Hospitals. All were subjected to full medical history emphasizing the duration of diabetes mellitus and the type of treatment, along with thorough clinical examination including blood pressure and anthropometric measurements (Height, weight, Body Mass Index (BMI) and Waist/Hip ratio). Subjects with history of impaired renal function, impaired liver function, heart failure, cancer, autoimmune disease, pregnancy, and alcohol or drug abuse were excluded. Also, patients using a dipeptidyl peptidase-4 inhibitor or a GLP-1 agonist therapy were excluded.

BMI was calculated as body weight in kilograms divided by the height in meters squared (kg/m^2) and waist circumference was measured at the highest point of the iliac crest at minimal respiration to the nearest 0.1 cm. Serum lipid concentrations were assayed using Quantitative Enzymatic Colorimetric Determination for total and HDL cholesterol and triglycerides in plasma (Stanbio Cholesterol Liquicolor, Procedure NO. 1010). LDL cholesterol was calculated using the Friedewald equation as follows: LDL-C = (Total cholesterol- HDL-C) + Triglycerides/5[9]. Fasting blood glucose (FBG) and 2hPP plasma glucose were measured using an automated glucose oxidase method using Behring Diagnostics Reagents (SVR Glucose Test; Behring, La Jolla, CA). HbA1c is assayed by Stanbio Procedure No.0350 “Quantitative colorimetric determination of Glycohemoglobin in blood”. Enzyme linked immunoassay (ELISA) was used for in vitro quantitative measurements of fasting plasma insulin (BioSource INS-EASIA Kit. Catalogue number: KAP1251). Insulin resistance was estimated by HOMA-IR and was defined as fasting serum insulin (μU/ml)×FPG (mmol/l)/22.5[10].

Sample Collection: Subjects were instructed to fast 8 hours, 7 ml of venous blood was collected by venipuncture under complete aseptic conditions, and then the subjects were instructed to continue fasting. After completing 14 hours of fasting, another 3 ml of venous blood were collected. The first sample was used for measurement of FPG, fasting insulin,

fasting GLP-1 and HbA_{1c}. The second sample was used for measurement of lipid profile (total cholesterol, HDL-C, LDL-C and triglycerides), and it was frozen at -20°C until assayed. Next, 75 gm glucose were ingested by all subjects after the second venipuncture then two hours later another 3 ml of venous blood were collected to measure two hours post prandial plasma glucose (2hPPG) and 2hPPGLP-1. Samples that were used for estimation of GLP-1 were stored at -70 C° for subsequent assay in plasma using DRG Glucagon like peptide-1 (Human,Rat and Mouse) ELISA (EIA-4141) kit, USA (Porstmann and Kiessig,1992)[11].

Surgical Technique: The technique used in this study was RYGB, which combines restrictive and malabsorptive mechanisms. A vertical gastric pouch (20–30 ml) was constructed with surgical staples in the lesser curvature of the stomach. Gastrojejunostomy adjustment was performed using a 32-G French tube. Reconstruction was performed by RYGB with an alimentary limb measuring 100 cm and a biliopancreatic limb of 50 cm from the ligament of Treitz.

Statistical Analysis:

Data analysis was performed using the SPSS program, v.12. Data were expressed as mean ± standard deviation (SD) for parametric data and as median and interquartile range (IQR) for non-parametric data respectively. Parametric data were analyzed using one-way analysis of variance (ANOVA) for the comparison of three groups. Pearson's correlation coefficient (r) test was used for correlating data. Independent-samples T test of significance was used when comparing between two groups. Statistical significance was detected at p value <0.05, while p <0.001 was accepted as highly significant.

Results:

The studied groups were matched regarding age and gender, and there was no statistically significant difference between group I and group II regarding diabetes duration and the use of anti-diabetic medications (Table 1). There was a highly statistical significant difference between group I and both group II and group III regarding BMI, while there was no statistical difference between (group II & group III). Regarding fasting and 2hPP GLP-1 levels, there were highly statistical significant differences among the three groups (table 1). Fasting GLP-1 was lower in obese diabetics than lean diabetics (4.33 ± 1.10 versus 5.21 ± 0.896 ng/ml, p=0.012), as well as 2hrsPP GLP-1 levels (4.94±1.17 versus 6.04±0.995 ng/ml, p= 0.006). All criteria of subjects included in the study are summarized in (table 1).

We also found a highly significant negative correlation between both fasting as well as 2 hours postprandial GLP-1 and all of the following: BMI, waist/ hip ratio, blood pressure, FPG, 2hPPG, HbA1c, fasting insulin, HOMA IR, total cholesterol, triglycerides and LDL-C, as well as the duration of diabetes mellitus. There was a highly significant positive correlation between fasting GLP-1 and HDL-C (Table 2).

Regarding the postoperative results, we found an increase in the fasting GLP-1 levels (from 4.3±1.1 to 4.53±1.02 ng/ml), not reaching statistical significance (p=0.095), while there was a highly statistical significant increase in the 2hPP GLP-1 level (from 4.9±1.2 to 9.79±1.99ng/ml, p<0.001). Also, there was a highly statistical significant decrease in BMI, waist/hip ratio, SBP, DBP, FPG, 2hPPG, HbA1c, fasting insulin, HOMA-IR, total cholesterol, triglycerides and LDL-C, while levels of HDL-C were increased significantly (Table 3).

BMI decreased significantly after surgery (from 47.3±4.2 to 42.10±3.06kg/m2 p<0.001). However, there was still a highly statistical significant difference between group I and both groups II and III. FPG, 2hPPG, fasting insulin, HOMA-IR, total cholesterol, triglycerides and LDL-C were significantly decreased to levels similar to healthy controls. After surgery, fasting GLP-1 levels were still low in comparison to the other 2 groups (4.53±1.02 vs. 5.21 ± 0.89, 6.14 ± 0.47 ng/ml, p < 0.001). The 2hPP GLP-1 levels were elevated to levels similar to healthy controls (9.79±1.999 vs. 9.32±0.97 ng/ml, p>0.05); (Table 4).

Our results also demonstrated a highly statistical significant reduction regarding the percentage of patients using anti-diabetic medications; as all patients (100%) were on treatment preoperatively, while only 40% were receiving anti-diabetic medications postoperatively. There was a highly significant decrease in use of insulin plus oral therapy from 45% to only 10%, as well as a reduction in the percentage of patients who use oral medications from 55% to 30% (Figure 1). There was also a statistical significant decrease in the use of anti-hypertensive and lipid lowering medications (Figure 2).

Table (1): Comparison among the three studied groups regarding the baseline results of different variables:

Variables		Group I (pre-op.)	Group II	Group III	ANOVA		Tukey's test	
		Mean ± SD	Mean ± SD	Mean ± SD	f	P-value	Comp.	P-value
Age (years)		46.10 ±4.84	45.45±5.48	44.60±4.50	0.299	0.743	-----	-----
SBP (mmHg)		138.75±7.59	127.25 ± 8.66	120.00±4.08	23.562	<0.001**	I&II	<0.001**
							I&III	<0.001**
							II&III	0.043*
DBP (mmHg)		90.75±4.06	82.50±7.695	75.50± 4.97	23.634	<0.001**	I&II	<0.001**
							I&III	<0.001**
							II&III	0.011*
BMI (kg/m²)		47.30±4.19	22.50±1.28	21.30±1.34	470.25	<0.001**	I&II	<0.001**
							I&III	<0.001**
							II&III	0.526
WHR		0.97±0.02	0.87±0.03	0.74±0.02	335.35	<0.001**	I&II	<0.001**
							I&III	<0.001**
							II&III	<0.001**
FPG (mg/dl)		145.85±19.19	136.40±17.12	85.30±6.60	46.843	<0.001**	I&II	0.181
							I&III	<0.001**
							II&III	<0.001**
2hPP.PG (mg/dl)		208.9±33.19	183.6±51.33	122.0±7.44	16.600	<0.001**	I&II	0.111
							I&III	<0.001**
							II&III	0.001*
HbA1c (%)		9.57±1.51	7.90±1.48	5.300±0.61	33.352	<0.001**	I&II	0.001*
							I&III	<0.001**
							II&III	<0.001**
Fasting Insulin (mU/ml)		20.90±6.84	13.00±2.13	5.96±0.53	38.199	<0.001**	I&II	<0.001**
							I&III	<0.001**
							II&III	0.001*
HOMA IR		7.83±3.61	4.46±1.28	1.26±0.21	25.564	<0.001**	I&II	<0.001**
							I&III	<0.001**
							II&III	0.004*
Total Cholesterol (mg/dl)		220.75±34.82	195.10±27.21	149.20±17.00	20.221	<0.001**	I&II	0.020*
							I&III	<0.001**
							II&III	0.001*
Triglycerides (mg/dl)		199.35±53.73	172.55±52.56	103.70±16.71	13.114	<0.001**	I&II	0.197
							I&III	<0.001**
							II&III	0.002*
HDL- Chol. (mg/dl)		34.10±9.36	39.25±5.02	59.20±7.269	38.799	<0.001**	I&II	0.085
							I&III	<0.001**
							II&III	<0.001**
LDL- Chol. (mg/dl)		173.92±26.82	152.74±18.05	116.62±15.15	23.522	<0.001**	I&II	0.009*
							I&III	<0.001**
							II&III	<0.001**
F GLP-1 (ng/ml)		4.33±1.10	5.21 ±0.896	6.14±0.47	13.250	<0.001**	I&II	0.012*
							I&III	<0.001**
							II&III	0.033*
2hPP. GLP-1 (ng/ml)		4.94±1.17	6.04 ±0.995	9.320±0.97	57.105	<0.001**	I&II	0.006*
							I&III	<0.001**
							II&III	<0.001**
Duration of DM (years)		4.78±1.86	4.38±1.597	---------	**T-Test**		**t**	0.730
							P-value	0.470
Gender N (%)	**Male** 16 (32%)	5 (25%)	7 (35%)	4 (40%)	**Chi-Square**		**X^2**	0.835
	Female 34 (68%)	15 (75%)	13 (65%)	6 (60%)			**P-value**	0.659
Antidiabetic medications N (%)	**Insulin+oral**	9 (45%)	7 (35%)	---------	**Chi-Square**		**X^2**	0.104
	Oral only	11 (55%)	13 (65%)				**P-value**	0.746

Variables		Group I (pre-op.)	Group II	Group III	ANOVA		Tukey's test	
		Mean ± SD	Mean ± SD	Mean ± SD	f	P-value	Comp.	P-value
Age (years)		46.10 ±4.84	45.45±5.48	44.60 ± 4.50	0.299	0.743	-----	-----
SBP (mmHg)		138.75±7.59	127.25±8.66	120.00±4.08	23.562	<0.00**	I&II	<0.001**
							I&III	<0.001**
							II&III	0.043*
DBP (mmHg)		90.75±4.06	82.50±7.695	75.50 ± 4.97	23.634	<0.001**	I&II	<0.001**
							I&III	<0.001**
							II&III	0.011*
BMI (kg/m²)		47.30±4.19	22.50±1.28	21.30 ± 1.34	470.25	<0.001**	I&II	<0.001**
							I&III	<0.001**
							II&III	0.526
WHR		0.97±0.02	0.87±0.03	0.74 ± 0.02	335.35	<0.001**	I&II	<0.001**
							I&III	<0.001**
							II&III	<0.001**
FPG (mg/dl)		145.85±19.19	136.40±17.12	85.30 ± 6.60	46.843	<0.001**	I&II	0.181
							I&III	<0.001**
							II&III	<0.001**
2hPP.PG (mg/dl)		208.9±33.19	183.6±51.33	122.0± 7.44	16.600	<0.001**	I&II	0.111
							I&III	<0.001**
							II&III	0.001*
HbA1c (%)		9.57±1.51	7.90±1.48	5.300 ± 0.61	33.352	<0.001**	I&II	0.001*
							I&III	<0.001**
							II&III	<0.001**
Fasting Insulin (mU/ml)		20.90±6.84	13.00±2.13	5.96± 0.53	38.199	<0.001**	I&II	<0.001**
							I&III	<0.001**
							II&III	0.001*
HOMA IR		7.83±3.61	4.46±1.28	1.26± 0.21	25.564	<0.001**	I&II	<0.001**
							I&III	<0.001**
							II&III	0.004*
Total Cholesterol (mg/dl)		220.75±34.82	195.10±27.21	149.20±17.00	20.221	<0.001**	I&II	0.020*
							I&III	<0.001**
							II&III	0.001*
Triglycerides (mg/dl)		199.35±53.73	172.55±52.56	103.70±16.71	13.114	<0.001**	I&II	0.197
							I&III	<0.001**
							II&III	0.002*
HDL- Chol. (mg/dl)		34.10±9.36	39.25±5.02	59.20± 7.269	38.799	<0.001**	I&II	0.085
							I&III	<0.001**
							II&III	<0.001**
LDL- Chol. (mg/dl)		173.92±26.82	152.74±18.05	116.62±15.15	23.522	<0.001**	I&II	0.009*
							I&III	<0.001**
							II&III	<0.001**
F GLP-1 (ng/ml)		4.33±1.10	5.21 ±0.896	6.14 ± 0.47	13.250	<0.001**	I&II	0.012*
							I&III	<0.001**
							II&III	0.033*
2hPP. GLP-1 (ng/ml)		4.94±1.17	6.04 ±0.995	9.320 ± 0.97	57.105	<0.001**	I&II	0.006*
							I&III	<0.001**
							II&III	<0.001**
Duration of DM (years)		4.78±1.86	4.38±1.597	---------	**T-Test**		**t**	0.730
							P-value	0.470
Gender N (%)	**Male** 16 (32%)	5 (25%)	7 (35%)	4 (40%)	**Chi-Square**		**X^2**	0.835
	Female 34(68%)	15 (75%)	13 (65%)	6 (60%)			**P-value**	0.659
Antidiabetic medications N(%)	**Insulin+oral**	9 (45%)	7 (35%)	--------	**Chi-Square**		**X^2**	0.104
	Oral only	11 (55%)	13 (65%)				**P-value**	0.746

Group I: obese T2DM **Group II:** Lean T2DM **Group III:** Lean healthy control

Table (2): Correlation between fasting GLP-1, 2hsPP GLP-1 levels and the different variables:

Correlations				
Variables	**F GLP-1 (ng/ml)**		**2hPP GLP-1 (ng/ml)**	
	R	**P-value**	**r**	**P-value**
Age (years)	0.031	0.897	-0.002	0.994
Duration of diabetes(years)	-0.981	<0.001**	-0.693	<0.001**
SBP (mmHg)	-0.819	<0.001**	-0.745	<0.001**
DBP (mmHg)	-0.662	0.001*	-0.644	0.002*
BMI (kg/m²)	-0.818	<0.001**	-0.742	<0.001**
WHR	-0.754	<0.001**	-0.670	<0.001**
FPG (mg/dl)	-0.823	<0.001**	-0.731	<0.001**
2hPP.PG (mg/dl)	-0.792	<0.001**	-0.752	<0.001**
HbA1c (%)	-0.833	<0.001**	-0.760	<0.001**
Fastig insulin (mU/ml)	-0.832	<0.001**	-0.760	<0.001**
HOMA IR	-0.826	<0.001**	-0.756	<0.001**
Total Cholest (mg/dl)	-0.806	<0.001**	-0.748	<0.001**
Triglycerides (mg/dl)	-0.825	<0.001**	-0.752	<0.001**
HDL-Chol. (mg/dl)	0.807	<0.001**	0.721	<0.001**
LDL-Chol. (mg/dl)	-0.773	<0.001**	-0.726	<0.001**

***** Significant difference , ****** highly significant difference.

Table (3): Comparison between the preoperative and 3 months post-RYGP results in obese T2D:

Variables	**Pre-operative**	**Post-operative**	**Paired Differences**		**Paired t-test**	
	Mean ± SD	**Mean ± SD**	**Mean**	**SD**	**t**	**P-value**
SBP (mmHg)	138.8 ± 7.6	123.750 ± 8.252	15.000	2.294	29.240	<0.001**
DBP(mmHg)	90.8 ± 4.1	83.000 ± 8.176	7.750	5.250	6.601	<0.001**
BMI(kg/m²)	47.3 ± 4.2	42.100 ± 3.059	5.200	1.508	15.422	<0.001**
WHR	0.97±0.02	0.911 ± 0.029	0.060	0.016	17.245	<0.001**
FPG(mg/dl)	145.9 ± 19.2	98.250 ± 16.032	47.600	6.151	34.609	<0.001**
2hPP.PG(mg/dl)	208.9 ± 33.2	137.850 ± 14.908	71.050	20.715	15.339	<0.001**
HbA1c (%)	9.6 ± 1.5	7.110 ± 1.770	2.460	0.339	32.419	<0.001**
F. insulin (mU/ml)	20.9 ± 6.8	7.250 ± 1.997	13.650	4.902	12.453	<0.001**
HOMA IR	7.8 ± 3.6	1.829 ± 0.806	5.998	2.815	9.527	<0.001**
T.Cholest(mg/dl)	220.8 ± 34.8	173.250 ± 32.197	47.500	11.678	18.191	<0.001**
TG(mg/dl)	199.4 ± 53.7	143.950 ± 42.465	55.400	14.926	16.599	<0.001**
HDL-Chol.(mg/dl)	34.1 ± 9.4	48.050 ± 10.870	-13.950	2.685	-23.237	<0.001**
LDL-Chol.(mg/dl)	173.9 ± 26.8	135.000 ± 26.253	38.920	12.159	14.315	<0.001**
F GLP-1(ng/ml)	4.3 ± 1.1	4.525± 1.02	-0.195	0.150	-2.025	0.095
2hPPGLP1(ng/ml)	4.9 ± 1.2	9.785 ± 1.999	-4.845	0.842	-25.734	<0.001**

***** Significant difference, ****** highly significant difference.

Table (4): Comparison among the three studied groups 3 months after RYGB regarding the different variables:

Variables	Group I (post-operative)	Group II	Group III	ANOVA		Tukey's test	
	Mean ± SD	Mean ± SD	Mean ± SD	f	P-value	Comp.	P-value
SBP (mmHg)	123.75±8.25	127.25±8.66	120.00±4.08	2.987	0.060	I&II	0.340
						I&III	0.436
DBP (mmHg)	83.0±8.18	82.50±7.69	75.50±4.97	3.798	0.030*	I&II	0.976
						I&III	0.033*
BMI (kg/m²)	42.10±3.06	22.50±1.28	21.300±1.337	502.56	<0.001**	I&II	<0.001**
						I&III	<0.001**
WHR	0.91±0.03	0.87±0.03	0.74±0.02	124.25	<0.001**	I&II	<0.001**
						I&III	<0.001**
FPG (mg/dl)	98.25±16.03	136.40±17.12	85.30±6.60	49.327	<0.001**	I&II	<0.001**
						I&III	0.081
2hPP.PG (mg/dl)	137.85±14.91	183.60±51.33	122.00±7.44	14.125	<0.001**	I&II	<0.001**
						I&III	0.460
HbA1c (%)	7.11±1.77	7.90±1.45	5.30±0.61	10.333	<0.001**	I&II	0.220
						I&III	0.008*
Fasting Insulin (mU/ml)	7.25±1.99	13.00±2.128	5.96±0.53	67.133	<0.001**	I&II	<0.001**
						I&III	0.187
HOMA IR	1.83±0.81	4.462±1.28	1.26±0.21	52.370	<0.001**	I&II	<0.001**
						I&III	0.294
Total Chol. (mg/dl)	173.25±32.19	195.10±27.21	149.20±17.00	9.409	<0.001**	I&II	0.043*
						I&III	0.076
Triglyceride (mg/dl)	143.95±42.47	172.55±52.56	103.70±16.71	8.421	0.001*	I&II	0.106
						I&III	0.054
HDL- Chol. (mg/dl)	48.05±10.87	39.25±5.01	59.20±7.27	19.903	<0.001**	I&II	0.004*
						I&III	0.003*
LDL- Chol. (mg/dl)	135.00±26.25	152.74±18.05	116.62±15.15	10.003	<0.001**	I&II	0.030*
						I&III	0.077
F GLP-1 (ng/ml)	4.525±1.02	5.21±0.896	6.14±0.47	11.214	<0.001**	I&II	0.048*
						I&III	<0.001**
2hPP. GLP-1 (ng/ml)	9.79±1.999	6.04±0.995	9.32±0.97	35.529	<0.001**	I&II	<0.001**
						I&III	0.699

***** Significant difference, ****** highly significant difference.
Group I: obese T2DM Group II: Lean T2DM Group III: Lean healthy control

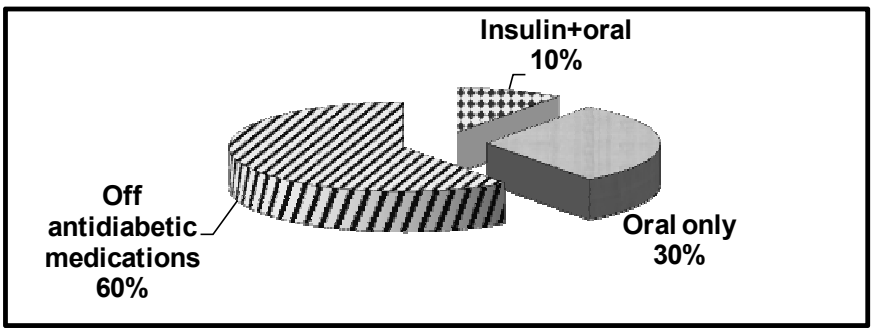

Figure (1): Descriptive figure for the percentage of usage of the anti-diabetic medications among obese T2DM 3 months post- RYGB surgery

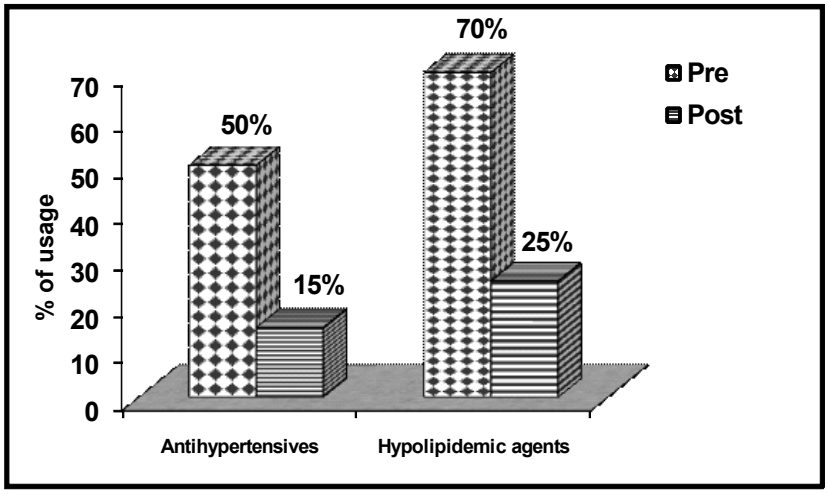

Figure (2): Comparison between the preoperative and postoperative use of antihypertensive and hypolipidemic medications in obese type 2 diabetics.

Discussion:

The results of our study showed a highly statistically significant decrease of the fasting and 2hPPGLP-1 levels among type 2 diabetic patients compared with those of control subjects. These results were in agreement with those of Toft-Nielsen et al.[12] and Vilsboll et al.[13] who reported lower postprandial GLP-1 levels in patients with type 2 diabetes compared with normal oral glucose–tolerant subjects. However, some authors pointed out that the GLP-1 levels were not reduced in patients with type 2 diabetes mellitus in comparison to control subjects[14,15].

We found that fasting and 2hPP GLP-1 levels among obese diabetic patients were significantly lower than those of lean diabetics. This was supported by our finding of a negative correlation between GLP-1 levels and body mass index, which was of a high statistical significance (p<0.001).These results were in agreement with those of Toft-Nielsen et al.[12] and Vollmer et al.[15] who found a decreased GLP-1 response to oral glucose load with increasing BMI. Furthermore, the fasting GLP-1 concentrations were found to be inversely related to BMI (P = 0.003) by Greenfield et al.[16] On the other hand, Yamaoka-Tojo et al.[17] found a statistically non significant positive correlation between fasting GLP-1 concentrations and BMI (P = 0.747), and in the study of Lee et al.[18] the postprandial GLP-1 levels were not correlated with BMI. Moreover, Kozawa et al.[19] showed that incretin secretion did not differ between Japanese obese and non-obese patients with type 2 diabetes. They explained their results as the decreased secretion of GLP-1 in Caucasian subjects with T2DM may be related to insulin resistance and obesity[20], but the obesity and insulin resistance in their patients did not reach levels sufficient to influence GLP-1 secretion. Alternatively, it may be that total GLP- 1 levels in Japanese subjects are naturally low compared with those in Caucasian subjects and not parallel to insulin secretion[19].

The results of our study showed a highly statistical significant negative correlation between both fasting and 2hPP GLP-1 levels and FBG, HbA1c, fasting insulin and HOMA IR (p<0.001). Zhang et al.[21] found that both fasting and postprandial GLP-1 levels were reduced in type 2 diabetic patients compared to subjects with normal glucose tolerance (P<0.005), and their levels were inversely proportional with the HOMA-IR. On the other hand, Lee et al.[18] examined Japanese newly diagnosed T2DM patients with relatively mild hyperglycemia (HbA1c levels of less than 7.5%), none of which were receiving any glucose-lowering medications. The authors demonstrated that, although GLP-1 was lower in the T2DM group than in the NGT group at 120 minutes in the OGTT, there were no statistical significant differences, and GLP-1 levels were not correlated with FPG, HbA1c or HOMA-IR. Although the cause and the mechanism for this discrepancy are not clear until now, the possible factors for it may be: course of disease, sample size and influence of treatment[21].

We also found that GLP-1 concentrations were inversely related to SBP, DBP, BMI, total cholesterol, LDL-cholesterol and triglyceride levels but directly related to HDL- cholesterol, all the previous correlations were of a highly statistical significant importance (p<0.001). These results were in line with Zhang et al.[21] who showed that the total fasting and postprandial GLP-1 levels were positively correlated with the HDL-C. Also, de Luis et al.[22] revealed a significant negative correlation among serum GLP-1 levels and the independent variables; waist-to-hip ratio, glucose, total cholesterol, and LDL-cholesterol. They revealed that obese patients with metabolic syndrome had lower mean GLP-1 levels than those without metabolic syndrome and they found that GLP-1 levels remained as a preventive factor to develop metabolic syndrome.

There was a postoperative highly significant decrease in systolic blood pressure, diastolic blood pressure, BMI, waist/hip ratio, FBG & 2hPP, HbA1c, fasting insulin and HOMA IR (p<0.001).These results were in agreement with those of Mor´inigo et al.[23] and Laferrère et al.[24] who reported that 6 weeks following RYGB, there was a significant decrease in the systolic blood pressure, diastolic blood pressure, BMI, waist circumference, fasting glucose, fasting insulin and HOMA-IR.

In our study, there was a postoperative increase in the fasting GLP-1 level but statistically insignificance, while there was a highly statistical significant increase in the 2hours postprandial GLP-1 levels. Our results were in agreement with those of Laferrère et al.[24] and Kashyap et al.[25] who showed that fasting GLP-1 was not altered by surgery, however, the postprandial GLP-1 response was increased (P<0.05). In addition, Umeda et al.[26] observed early changes occurring in the shape of GLP-1 postprandial curve thirty days after RYGB, and they suggested that the RYGB surgery induces early beneficial hormonal changes and is a very efficient surgical therapy for rapid glycemic control in obese patients with type 2 diabetes.

We found that 60% of patients have stopped their anti-diabetic medications, with a decrease in use of insulin plus oral therapy from 45% to only 10%, as well as a reduction in the percentage of patients who use oral anti-diabetics from 55% to 30%. These results were in agreement with Hall et al.[27] Who found that diabetes remission is achieved in 68.4% of obese subjects who underwent RYGB, a reduction in insulin ± oral anti-diabetics from 26.5% to 8.2% and reduction in oral anti-diabetics use from 73.5% to 23.5%. There was also a statistical significant decrease in the percentage of use of antihypertensive medications (from 50% to 15%) and lipid lowering medications (from 70% to 25%), (P<0.05). These findings were consistent with Mingrone et al.[28] demonstrated 75% diabetes remission, and they showed that the antihypertensive therapy was reduced in 80% of patients who underwent RYGB.

Conclusion:

RYGB surgery resulted in significant reduction in BMI and improvement in all metabolic parameters. 2hPP GLP-1 is low in diabetic patients. Postoperative 2hPP GLP-1 levels increased, possibly responsible for the metabolic benefits. Diabetes remission was noted in 60% of obese diabetic patients after surgery.

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Original Article

Correlation Between Abnormal Liver Enzymes and Serum Adiponectin Level in Obese Type 2 Diabetic Patients.

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Abstract:

Background: Adiponectin seems to be secreted as a protective response to increased levels of pathologic adipocytokines and hypoadiponectinaemia is implicated in the pathogenesis of non-alcoholic fatty liver disease (NAFLD), insulin resistance and the metabolic syndrome (MetS). Patients with obesity and co-morbid type 2 diabetes mellitus (T2DM) are at increased risk for NAFLD and cirrhosis compared to obese nondiabetic patients. **Aim:** This work was done to correlate between serum adiponectin level and abnormal liver enzymes (ALT and AST) in obese type 2 diabetic patients. **Patients:** Thirty obese type 2 diabetic patients (as patients group) and ten non diabetic obese subjects (as a control group) were included. Thorough Clinical examination was done, serum ALT, AST, fasting and 2 hours postprandial blood glucose, C-reactive protein , uric acid , HbA1c, s. triglycerides, s. cholesterol, s. HDL-cholesterol and s. adiponectin were measured. **Results:** Serum adiponectin was significantly lower in type 2 diabetic obese patients than non diabetic obese control subjects. Within the patient group serum adiponectin was significantly higher in female than male among obese type 2 diabetic patients, serum adiponectin correlated negatively with Alanine transaminase (ALT), Aspartat transaminase, age, systolic BP, diastolic BP, body mass index(BMI), W/H ratio, fasting blood glucose ,2 hours post prandial blood glucose, C-reactive protein , HbA1c, Uric acid, s. triglycerides and s. cholesterol in type 2 diabetic obese patients. But it correlated positively with HDL-cholesterol. **Conclusion:** Hypoadiponectimia is associated with NAFLD in type2 diabetic obese Egyptian patients especially among obese males.

Keywords: adiponectin, BMI, insulin resistance, MetS and NAFLD

Introduction:

Metabolic syndrome (MetS) refers to a condition associated with insulin resistance in which three of five signs must be present, including abdominal obesity, impaired fasting glucose, elevated blood pressure, hypertriglyceridemia and low HDL cholesterol**,** in addition to the classic five signs mentioned above, the metabolic syndrome is associated with a number of other conditions, including microalbuminuria, fatty liver, endothelial dysfunction, hyperuricaemia and systemic inflammation.[1]

Adiponectin is a hormone secreted by Adipocytes. It is found in relatively high circulating levels in plasma but is decreased in patients with NAFLD and in clinical states associated with insulin resistance such as MetS and type 2 diabetes mellitus**.** It regulates energy homeostasis and glucose and lipid metabolism.[2]

A low serum level of adiponectin has been found to be an independent risk factor for the development of hypertension in both cross sectional and prospective studies. Serum levels of adiponectin are significantly lower in individuals with essential hypertension than in normotensive healthy individuals, even after adjustment for confounding factors.[3]

Plasma adiponectin levels have been reported to be reduced in obese humans, particularly those with visceral obesity, and to correlate inversely with insulin resistance. Prospective and longitudinal studies have shown that lower adiponectin levels are associated with a higher incidence of diabetes.[4]

In our work serum adiponectin concentration was estimated in thirty obese type 2 diabetic patients and ten matched age and gender obese non diabetic as a control group from outpatient clinic.

Patients and Methods:

1-Patients:

This cross sectional study was conducted on 40 subjects from the outpatient clinic of Kasr El Aini Hospital. Faculty of Medicine. Cairo University. A written informed consent was obtained from all eligible patients. The research protocol was approved by the local university review committee.

These patients were categorized into:

Group 1: 30 obese type 2 diabetic patients

Group 2: 10 matched age and gender obese non diabetic subjects (as a control group)

All the patients included in the study were subjected to full history and thorough clinical examination. Parameters were obtained as age, gender, blood pressure (BP) in mmHg, weight (WT) in kg, height (HT) in cm, body mass index (BMI) in Kg/m^2, (WC) in cm, hip circumference (HC) in cm, waist / Hip ratio (W/H)

2- Investigations:

The patients were subjected to the following investigations:

1- Serum Alanine transaminase (ALT)

2- Serum Aspartat transaminase (AST)

3- Fasting blood glucose, 2 hours postprandial blood glucose

4- Serum uric acid (UA)

5- C-reactive protein (CRP)

6- S. Triglycerides, S. Total cholesterol, HDL-cholesterol (HDL).

7- Hemoglobin A1c (HbA1c)

8- Total serum adiponectin will be measured using Enzyme Linked Immune Sorbant Assay (ELISA) technique

NB: Fasting adiponectin in obese non diabetic subjects (as a control group) {4400-4700ng/ml in female} and {3700-3900ng/ml in male}.

All blood samples were drawn after 12 hours an overnight fast. Plasma samples were kept at - 70°C for subsequent assays.

Estimation of Serum adiponectin:

Adiponectin was assayed using ELISA technique AviBion Human Adiponectin (Acrp30) ELISA Kit Orgenium Laboratories Viikinkaari 6 FIN-00790 Helsinki FINLAND. Datacollection Anthropometric data including age, sex, height, weight and WC were collected. Height was measured with subject standing without shoes by standard stadiometer and the nearest one centimeter. Weight was measured with subjects wearing light clothing, the nearest 0.5kilogram was recorded. WC was measured at the end of a normal expiration, midway between the inferior margin of the ribs and the superior boarder of the iliac crest in a horizontal plane. Body mass index (BMI; kg/m2) was collected according to the Quetelet equation (weight in kilograms divided by height in square meter)

Statistical Analysis:

Data were statistically described in terms of range, mean ± standard deviation (± SD), frequencies (number of cases) and percentages when appropriate. Comparison of quantitative variables between the study groups was done using student t test for independent samples. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. Correlation between various variables was done using Pearson moment correlation equation for linear relation. A probability value (p value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2003 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 16 for Microsoft Windows.

Results:

As shown in table 5 above, there was strong negative correlation between adiponectin and (Age, Systolic BP, Diastolic BP, BMI, Waist circumference and W/H ratio). There was a moderate negative correlation between adiponectin and (Height and Hip circumference). There was no significant correlation between adiponectin and weight.

As shown in table 6, there was strong negative correlation between adiponectin and (ALT, AST, Fasting blood glucose, 2 hours post

prandial blood glucose C-reactive protein, Triglycerides and HbA1c). There was moderate negative correlation between adiponectin and (Uric acid and Cholesterol). There was moderate positive correlation between adiponectin and HDL-cholesterol.

Table 1: Descriptive statistics of clinical data of patients included in the study.

Variable	Minimum	Maximum	Mean±SD
Age(years)	38	58	46.40 ±5.92
Systolic BP(mmHg)	110	190	144 ±23.13
Diastolic BP(mmHg)	70	110	91.33 ±11.67
Weight (Kg)	69	105	87.10 ±9.02
Height (cm)	145	175	160.60±9.08
BMI (kg/m^2)	31	38.7	33.59 ±2.27
Waist circumference(cm)	109	142	121.50±10.53
Hip circumference(cm)	104	115	109.17±3.15
W/H ratio	1.03	1.28	1.12 ±0.07

Table 2: Descriptive statistics of clinical data of control group included in the study

Variable	Minimum	Maximum	Mean±SD
Age(years)	40	55	47 ±5.46
Systolic BP(mmHg)	110	130	117±6.75
Diastolic BP(mmHg)	70	90	79±5.68
Weight (Kg)	70	101	86.1±8.87
Height (cm)	150	180	165.9±9.05
BMI(kg/m^2)	31	31.5	31.29±0.16
Waist circumference(cm)	96	121	111.8±8
Hip circumference(cm)	106	128	115.1±5.84
W/H ratio	0.90	1.05	0.97±0.06

Table 3: Comparison between descriptive statistics of clinical data of patients and control group included in the study.

Variable	Patients (30) Mean±SD	Control(10) Mean±SD	P-value	Significance
Age(years)	46.40 ±5.92	47 ±5.46	0.779	N Sig
Systolic BP(mmHg)	144 ±23.13	117±6.75	0.001	Sig
Diastolic BP(mmHg)	91.33 ±11.67	79±5.68	0.003	Sig
Weight (Kg)	87.10 ±9.02	86.1±8.87	0.762	N Sig
Height (cm)	160.60±9.08	165.9±9.05	0.118	N Sig
BMI(kg/m^2)	33.59 ±2.27	31.29±0.16	0.000	Sig
Waist circumference(cm)	121.50±10.53	111.8±8	0.011	Sig
Hip circumference(cm)	109.17±3.15	115.1±5.84	0.000	Sig
W/H ratio	1.12 ±0.07	0.97±0.06	0.000	Sig

p-value is considered significant if < 0.05
Sig: significant
N Sig: non significant

Table 4: Comparison between descriptive statistics of investigations of patients and control group included in the study.

Variable	Patients(30) Mean±SD	Control(10) Mean±SD	P-value	Significance
ALT(u/l)	46.73±14.99	21 ±3.27	0.000	Sig
AST(u/l)	41.43±15.35	18.7±3.23	0.000	Sig
Fasting blood glucose (mg/dl)	134±35.91	80.3±4.64	0.000	Sig
Post prandial blood glucose (mg/dl)	194.80±48.66	107.5±12.51	0.000	Sig
Uric acid (mg/dl)	5.28±1.47	3.95±0.55	0.080	N Sig
C-reactive protein(mg/dl)	18.3±15.22	7.8±7.1	0.060	N Sig
Triglycerides(mg/dl)	168.63±39.14	110.30±7.42	0.000	Sig
Cholesterol (mg/dl)	213.57 ±43	188.2±40.23	0.109	N Sig
HDL-cholesterol(mg/dl)	41.2±8.62	45.2±6.6	0.189	N Sig
HbA1c %	6.29±0.57	5.96±0.34	0.090	N Sig
Adiponectin(ng/ml)	2693.3±863	4390±338.13	0.000	Sig

p-value is considered significant if < 0.05 Sig: Significant N Sig: Non significant

Table 5: Correlation between Adiponectin and clinical data of patients group included in the study

Variable	adiponectin in the patients		
	r	p-value	Significance
Age(years)	-0.810	0.000	Sig
Systolic BP(mmHg)	-0.814	0.000	Sig
Diastolic BP(mmHg)	-0.773	0.000	Sig
Weight (kg)	-0.293	0.116	N Sig
Height (cm)	-0.688	0.000	Sig
BMI(kg/m^2)	-0.862	0.000	Sig
Waist circumference(cm)	-0.854	0.000	Sig
Hip circumference(cm)	-0.688	0.000	Sig
W/H ratio	-0.832	0.000	Sig

p-value is considered significant if < 0.05 Sig: significant N Sig: non significant
r: is considered weak if < 0.25 , mild if ≥ 0.25- <0.5 , moderate if ≥ 0.5- <0.75 and strong if ≥ 0.75 .

Table 6: Correlation between Adiponectin and other investigations of patients group included in the study

Variable	adiponectin in the patients		
	r	p-value	Significance
ALT(u/l)	-0.943	0.000	Sig
AST(u/l)	-0.808	0.000	Sig
Fasting blood glucose (mg/dl)	-0.921	0.000	Sig
Post prandial blood glucose (mg/dl)	-0.877	0.000	Sig
Uric acid (mg/dl)	-0.699	0.000	Sig
C-reactive protein(mg/dl)	-0.806	0.000	Sig
Triglycerides (mg/dl)	-0.911	0.000	Sig
Cholesterol (mg/dl)	-0.537	0.002	Sig
HDL-cholesterol(mg/dl)	0.635	0.000	Sig
HbA1c %	-0.945	0.000	Sig

p-value is considered significant if < 0.05 Sig significant
r : is considered weak if < 0.25 , mild if ≥ 0.25- <0.5 , moderate if ≥ 0.5- <0.75 and strong if ≥ 0.75

Discussion:

It has been reported that the prevalence of obesity in adults is very high in Egypt, particularly among women, and that the prevalence of diabetes and hypertension parallels that of obesity. The overall prevalence of central obesity among Egyptian adults, according to the 2 indicators; WC and W/H ratio was 24.1% and 28.7% respectively. These figures are relatively high if the association of central obesity with morbidity and mortality is taken into consideration (Abolfotouh M A, et al.,2008).[5]

Adiponectin is a recently described adipokine that has been recognized as a key regulator of insulin sensitivity and tissue inflammation. It is produced by adipose tissue (white and brown) and circulates in the blood at very high concentrations. It has direct actions in liver, skeletal muscle and the vasculature, with prominent roles to improve hepatic insulin sensitivity, increase fuel oxidation [via up-regulation of adenosine monophosphate activated protein kinase (AMPK) activity] and decrease vascular inflammation (Whitehead J. P. , et al,.2006).[6]

Adiponectin seems to be secreted as a protective response to increased levels of pathologic adipocytokines as it possesses antidiabetic, antiatherogenic and anti-inflammatory properties (Kadowaki T et al., 2006).[7]

Hypoadiponectinemia has also been demonstrated to be independently associated with the metabolic syndrome indeed. Reduced plasma adiponectin levels are also commonly observed in a variety of states frequently associated with insulin resistance, such as cardiovascular disease and hypertension (Adamczak, M., et al. 2003).[8]

In our work serum adiponectin concentration was estimated in thirty obese type 2 diabetic patients with (24) females and (6) males (80% and 20% respectively) 70 % of them had fatty liver by abdominal U/S and ten (8 females and 2 males) matched age obese non diabetic as a control group.

There is a sexual dimorphism in the circulating levels of adiponectin, indeed, female humans have higher plasma adiponectin levels than males, suggesting that sexual hormones regulate the production of adiponectin, although it is controversial how these hormones, such as estrogen and testosterone, are involved in the regulation of plasma adiponectin level (Xu, A., et al. 2005)[9] Nevertheless, this may partially account for the fact that females are more sensitive to insulin than males.

In our work, there was negative correlation between Adiponectin and (Age, Systolic BP, Diastolic BP, Height, BMI, Hip circumference, Waist circumference and Waist/ Hip (W/H) ratio) in our 30 patients as shown in table 5. This was previously shown by Bilgili,et al.,2008[10] who stated that adiponectin levels were correlated negatively with BMI, waist and hip ratios, systolic and diastolic blood pressures. But they had shown that no significant correlation between adiponectin and age. Low serum level of adiponectin has been found to be an independent risk factor for the development of hypertension in both cross sectional and prospective studies. (Chow W-S et al. 2007)[3]

In our work, there was negative correlation between adiponectin and (ALT, AST) in our 30 patients as shown in table 6. In comparison Hickman, et al .2007[11] had shown that patients with obese T2DM are associated with decreased adiponectin and unexplained increases in liver enzymes and co-morbid type 2 diabetes with increased risk for NAFLD and cirrhosis compared to obese non diabetic patients (Hickman, et al .2007)[11]

Adiponectin has been shown to have a role in hepatic inflammation and steatosis. Hypo-adiponectinaemia is associated with NASH (Targher G, et al., 2004)[12] Adiponectin has been shown to have beneficial anti-inflammatory effects in liver, reducing steatosis, hepatomegaly and inflammation in mouse models of alcoholic and non-alcoholic fatty liver disease, in a study by Xu A. and his colleagues treatment with adiponectin decreased hepatomegaly, steatosis and alanine aminotransferase abnormality (Xu A, et al.,2003)[9]

It has also anti-inflammatory effect since it suppresses the hepatic production and plasma concentrations of TNF-α. In our work there was strong negative correlation between adiponectin and C-reactive protein in our 30 patients as shown in Table 6.

In agreement with our results, Devaraj et al. 2008[13] stated that, there was a significant negative correlation between CRP and adiponectin concentration. MetS seems to be a proinflammatory state characterized by increased concentrations of CRP, the inflammatory response correlates with multiple metabolic markers including obesity (particularly

visceral), dyslipidaemia, hypertension and insulin resistance. (Ouchi N, et al.,2003)[14]

Epidemiological studies reproducibly showed an inverse association between serum adiponectin concentrations and inflammatory markers and manifestations of the metabolic syndrome including CRP, fibrinogen, hypertension and endothelial function (Iwashima Y, et al., 2004)[15] In our work there was negative correlation between adiponectin and (Fasting blood glucose, 2 hours postprandial blood glucose Triglycerides and Cholesterol and uric acid) in patients. But there was positive correlation between adiponectin and HDL-cholesterol in our 30 patients as shown in Table 6.

In agreement with our results, Merja et al., 2006[16] stated that in both the sexes, adiponectin was correlated negatively with measures of body fat, fasting plasma glucose, 2 hours post prandial blood glucose, triglycerides and CRP. A positive correlation was found between adiponectin and fasting plasma insulin and HDL-cholesterol. Chedid R et al., 2010[17] stated that; serum uric acid was inversely correlated with adiponectin. Serum uric acid was positively correlated with BMI, WC, SBP, DBP, FPG, triglycerides, total and LDL-cholesterol, and HOMA index and inversely correlated with adiponectin ($p<0.001$ for all variables, $p<0.05$ for adiponectin). This study has few limitations, the small number of patients included in the study, lack of follow up for these patients, and absence of liver biopsy for staging the NASH and its correlation with adiponectin level

In conclusion serum adiponectin level are significantly lower in type 2 diabetic obese patients than non diabetic obese subjects, serum adiponectin level are significantly higher in female than male subjects in type 2 diabetic obese patients, serum adiponectin level correlate negatively with ALT and AST, age, systolic BP, diastolic BP, BMI and W/H ratio in type 2 diabetic obese patients. Also serum adiponectin level correlates negatively with fasting blood glucose, 2 hours postprandial blood glucose C-reactive protein, HbA1c, Uric acid, triglycerides and Cholesterol in type 2 diabetic obese patients. Finally serum adiponectin level correlate positively with HDL-cholesterol in type 2 diabetic obese patients. Finally, Hypoadeponectinemia is associated with NAFLD in type2 diabetic obese patients especially in obese males.

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Original Article

Vitamin D Status in Type 2 Diabetes Mellitus and Obesity; Lack of Impact on Metabolic and Inflammatory Markers.

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Abstract:

Introduction: Hypovitaminosis D is found to be highly prevalent worldwide. Obesity is an emerging endemic health problem, which is rapidly increasing in developing countries. Several studies have suggested that low vitamin D status contributes to insulin resistance and associated with markers of impaired glucose metabolism however, some studies reported that low dose vitamin D supplementation did not affect incident diabetes or parameters of glucose metabolism and hypovitaminosis D is not associated with increased prevalence of the metabolic syndrome. Our objective was to investigate the vitamin D serum level, intact parathormone (iPTH), sun exposure, C- reactive protein (CRP), fasting blood glucose (FBG) and hemoglobinA1c (HbA1c) in normal, obese, and diabetic subjects for clarifying the vit.D status in type 2 DM in relations to other variables. **Patient and Methods:** The study was carried out on 236 subjects who were divided into 3 groups; group 1, patients with type 2 DM, group 2, obese patients and group 3, age and sex matched apparently healthy subjects. Diabetes was defined by fasting glucose levels according to The American Diabetes Association guidelines. Any patient with history of vit.D supplementation, renal impairment, hypercalcaemia, rickets, osteomalasia, type 1 DM, pregnant or lactating females and patients receiving glucocorticoids were excluded. Measurement for 25 (OH) vit.D, iPTH, CRP, FBG, HbA1c and mean sun exposure (min/day) were measured for all participants. **Results:** A total of 52.97 % of participants were classified as vitamin D deficient (≤12ng/ml), 39.40% insufficient (20 - 12 ng/ml) and only 7.63 % of individuals had sufficient vitamin D (≥20 ng/ml). Group1 and 2 had significant higher levels of BMI, CRP, FBG & HbA1c and no significant differences as regard age, gender, vit.D, iPTH and sun exposure. There was no association between hypovitaminosis D and adiposity and there was no impact of hypovitaminosis D on glycaemic control or on markers of systemic inflammation. **Conclusion:** No relationship was found between hypovitaminosis D and obesity or glycaemic control in well-established type 2 DM. Future studies specifically designed to investigate the role of vitamin D on type 2 diabetes using inflammation as the main outcome are urgently needed in order to provide a more forceful link between vitamin D, inflammation and type 2 DM. Furthermore, genetic polymorphisms studies are also important in order to identify groups that are more susceptible to vitamin D deficiency and to developing type 2 diabetes in the population.

Keywords: Vitamin D, Type 2 Diabetes Mellitus, Obesity, Inflammatory Markers.

Introduction:

Hypovitaminosis D is found to be highly prevalent worldwide.[1] Accumulating evidence from several cross-sectional studies suggests that lower levels of circulating vitamin D are associated with an increased prevalence of type 2 diabetes mellitus (DM) and the metabolic syndrome[2]. Various definitions for vitamin D insufficiency have appeared in the literature; the best established one pertains to serum levels which are below 20ng/ml[1,3]. Interestingly, many studies have revealed that Vitamin D3 (calcitriol) has a role in the synthesis and the secretion of insulin[4] by receptor mediated molecular mechanisms[5]. Obesity is an emerging endemic health problem, which is rapidly increasing in developing countries[6]. A number of studies proved that the vitamin D3 precursor 7-dehydrocholesterol levels in the skin of obese people were not significantly different

from non obese people[7]. Several studies have suggested that low vitamin D status also contributes to insulin resistance[8]. Low vitamin D status is associated with markers of impaired glucose metabolism, such as glycosylated hemoglobin (HbA1c[9] some studies reported that low dose vitamin D supplementation did not affect incident diabetes or parameters of glucose metabolism in the predominantly Caucasian Women's Health Initiative cohort[10]. However, another study showed that within established type 2 diabetes, despite a common finding of vitamin D deficiency, hypovitaminosis D is not associated with increased prevalence of the metabolic syndrome. Neither is there any relationship between vitamin D levels and glycaemic control or cellular inflammation[11]. Observational studies have generated conflicting results. Some cross-sectional studies indicate that hypovitaminosis D is associated with higher serum levels of inflammatory biomarkers, such as IL-6, TNF-α, and C-reactive protein (CRP), in healthy [12,13]and in obese subjects[14], while others could not confirm these finding[15,16]. From the randomized controlled trials included, three of them used vitamin D alone, and again no convincing evidence that vitamin D supplementation have benefits on blood glucose control was observed. However, in most of these studies serum levels of 25(OH) vit.D in the patients, though lower than those of healthy controls were not actually "deficient". So, the question is raised if in the communities with the high prevalence of vitamin D insufficiency a significant difference in vitamin D status between normal, obese, and diabetic subjects still persists. So we plan to investigate the vit.D serum level, intact parathormone (iPTH), sun exposure, C- reactive protein(CRP), fasting blood glucose(FBG) and hemoglobin A1c (HbA1c) in normal, obese, and diabetic subjects for clarifying the vit.D status in type 2 DM in relations to other variables.

Patient and Methods:

The study was carried out in National Hospital, Riyadh, and KSA during Dec. 2011. The participants (236 subjects)were divided into 3 groups; group 1, patients with type 2 DM (76 patients; 42 males & 34 females) with mean age 46.16 ± 5.71 years,, group 2, obese patients (93 patients; 58 males & 35 females) with mean age 45.03±5.98 years,& group 3, age and sex matched apparently healthy subjects (67 subjects; 37males & 30 females). The diagnosis of T2D was identified from self-reported doctor's diagnosis and the reported use of hypoglycemic medication; subjects were also classified as diabetic if they reported use of diabetes medication without reporting a diagnosis of diabetes, and defining diabetes according to fasting glucose levels as defined in the American Diabetes Association guidelines[17]. Mean duration of diabetes was 4.19±2.25 years. BMI was calculated as (weight (kg)/height (meter^2)). The obesity was defined using guidelines[18]. Participants with BMI 18.5–24.9 kg/m^2 were classified as normal weight, BMI between 25-29.9 kg/m^2 were classified as overweight, and BMI ≥ 30 kg/m^2 were classified as obese. Any patient with history of vit.D supplementation, renal impairment, hypercalcaemia, rickets, osteomalasia, type1 DM, pregnant or lactating females and taking glucocorticoids were excluded. Measurement for 25 (OH) vit.D, iPTH, CRP, mean sun exposure (min/day), fasting blood glucose (FBG) & HbA1c were measured for all participants. The purpose of the study was described for all subjects and then a written informed consent was taken. Of all subjects, 5 mL of fasting blood sample was taken. After an hour at room temperature (RT), all samples were centrifuged at 2500 g at RT and then sera were transferred to fresh tubes in aliquots and kept at -70°C until the day of analysis. In our study; serum 25(OH) D levels were categorized as follows: sufficient, 20 ng/ml or more; insufficiency, from 20-12 ng/ml and deficiency less than 12 ng/ml[1].

Measurement of 25 (OH) vitamin D

The following investigations were performed:

1. 25 (OH) Vitamin D - Direct ELISA KIT (Immunodiagnostic)
2. Fasting Plasma blood glucose (GOD–POD method, Diatec kit, fully automated analyzer, XL – 300)
3. HbA1c (Immunoturbidimetric, direct technique, Futura system kit, by using an appropriate calibrator)
4. iPTH assay-Direct ELISA KIT (Immunodiagnostic)
5. Measurement of CRP typical immunoturbidimetric method (Roche)

Statistical Methods:

The Data were analyzed by computer using the statistical package SPSS for windows version 16 (software). Quantitative variables were reported as mean +/- SD, and qualitative variables as number and/or percentages. Comparing means was performed by independent samples T test. Correlations between different parameters were determined by bivariate Pearson correlation test. The linear relationship between variables was assessed by linear regression analysis. P values <0.05 were considered statistically significant.

Results:

Prevalence of vitamin D deficiency

25(OH) vit.D was quantified in a total of 236 participants with a mean age of 35.46±5.97 years. Among the participants, 58.10% were males, 32.20% had type 2 DM, 28.39% were normal weight, 9.32% were overweight and 62.26 % were obese. A total of 52.97 % of participants were classified as vitamin D deficient (<12 ng/ml), 39.40% insufficient (20 - 12 ng/ml) and only 7.63 % of individuals had sufficient vitamin D (>20 ng/ml) (p<0.001). Of particular note was the high prevalence of vitamin D deficiency in all participants (fig.1). Hypovitaminosis D had no gender prevalence.

Compared to the control group:

Group1 and 2 had significant higher levels of BMI, CRP, FBG & HbA1c (P < 0.001) and no significant differences as regard age, gender, vit.D, iPTH and sun exposure (P > 0.05) (tab.1).

In patients' groups:

Group 1 had significant higher levels of FBG & HbA1c (P <0.001) while group2 had significant higher levels of BMI (P< 0.001). Age, gender, vit.D, sun exposure and iPTH had no significant differences between both groups (P> 0.05).

By linear regression analysis (Univariate analysis), There was no association between hypovitaminosis D and adiposity and there was no impact of hypovitaminosis D on BMI, glycaemic control or on markers of systemic inflammation.

Correlations; By Pearson correlation, there was not any significant correlation between vit.D and other parameters in studied groups (1&2) (P >0.05 tab.2); in group 1, there was insignificant negative correlation between vit.D and mean age & CRP while there was insignificant positive correlation between vit.D and the mean of other variables(P > 0.05). In group 2, there was insignificant negative correlation between vit.D and mean BMI, FBG, HbA1c & CRP while there was insignificant positive correlation between vit.D and the mean of other variables (P > 0.05). In group 3, there was insignificant correlation between vit.D and mean BMI, FBG, HbA1c, CRP, iPTH and age (P > 0.05) (fig.2, 3, 4, 5, 6, 7).

Table1: Demographic and laboratory data of the studied groups (ANOVA test).

Parameters	Group 1 (Type 2 Dm)	Group 2 (Obese Cases)	Group 3 (Control)	P value
	Mean ± SD	Mean ± SD	Mean ± SD	
Age (year)	36.25 ± 6.25	35.03 ±5.98	35.25 ± 6.25	NS
Gender (male %)	55.26 %	62.36 %	55.2 %	NS
BMI (kg/m^2)	29.00 ± 5.71	32.50 ±1.85	20.89 ±1.76	< 0.001
FBG(mg/dl)	140.03 ± 3.62	86.46 ±1.09	83.24 ±1.27	<0.001
HbA1c (%)	8.77 ± 1.90	5.16 ± 0.27	5.00 ±0.24	< 0.001
CRP(mg/l)	3.83 ± 4.15	3.87 ± 3.25	2.63 ± 0.93	0.029
25(OH)vit.D (ng/ml)	11.94 ± 7.86	12.88 ± 7.96	11.83 ± 8.29	NS
iPTH(pmol/ml)	59.88 ± 22.00	58.57± 17.36	61.89 ±18.39	NS
Sun exposure(min)	10.44 ± 5.93	10.31 ± 5.66	11.01 ± 5.32	NS

FBG, fasting blood glucose; BMI, body mass index; HbA1c, hemoglobin A1c; CRP, C reactive protein; iPTH, intact parathormone.

Table 2: Pearson correlation between vit.D serum level and clinical & laboratory parameters among group1 & group2

Parameters	Group1 (type2DM)		Group 2 (obese cases)	
	Pearson correlation		Pearson correlation	
	Coefficient(r)	P value	Coefficient(r)	P value
Age (year)	-0.092	NS	0.146	NS
Gender (male %)	0.097	NS	0.108	NS
BMI (kg/m^2)	0.160	NS	-0.041	NS
FBG(mg/dl)	0.007	NS	-0.027	NS
HbA1c (%)	0.095	NS	-0.152	NS
CRP(mg/l)	-0.089	NS	-0.820	NS
iPTH(pmol/ml)	0.082	NS	0.091	NS
Sun exposure(min)	0.195	NS	0.070	NS

Fig. 1: Vitamin D status in studied groups

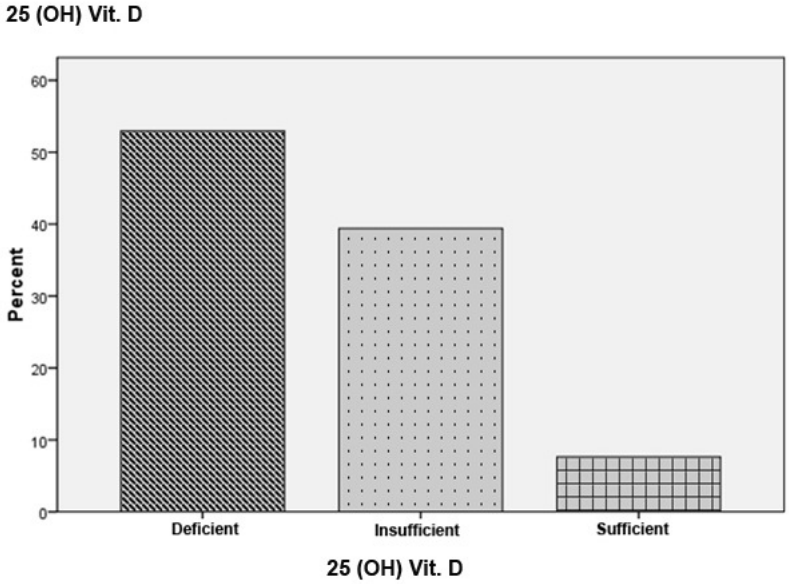


Fig. 2: Correlation between vit.D and BMI in obese group

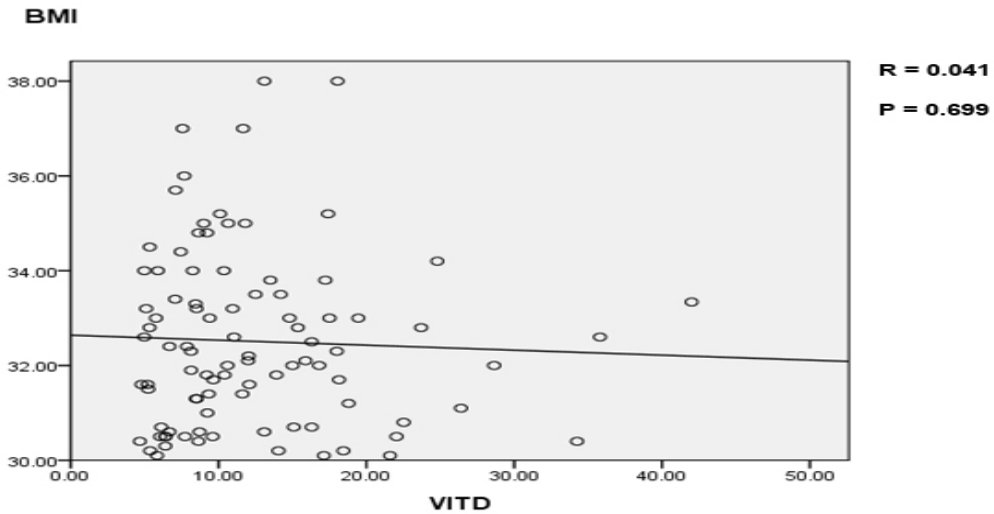


Fig. 3: Correlation between vit.D and BMI in diabetic group

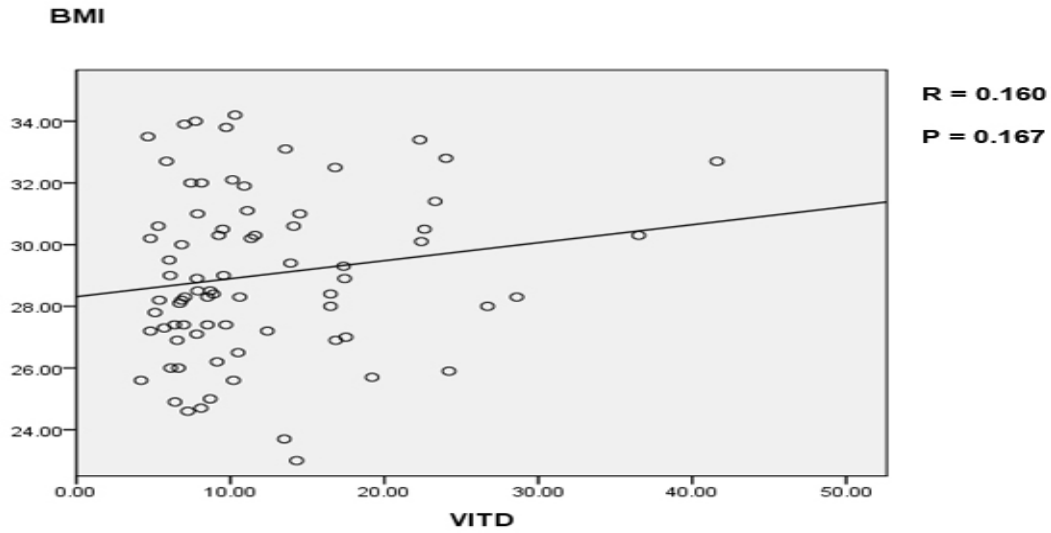


Fig 4: Correlation between vit.D and HbA1c in obese group

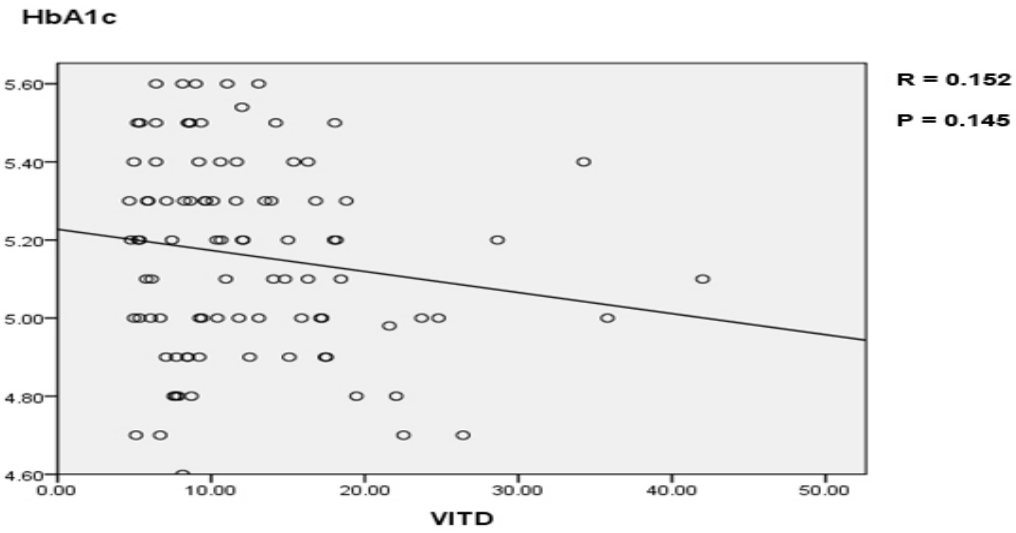


Fig 5: Correlation between vit.D and HbA1c in diabetic group

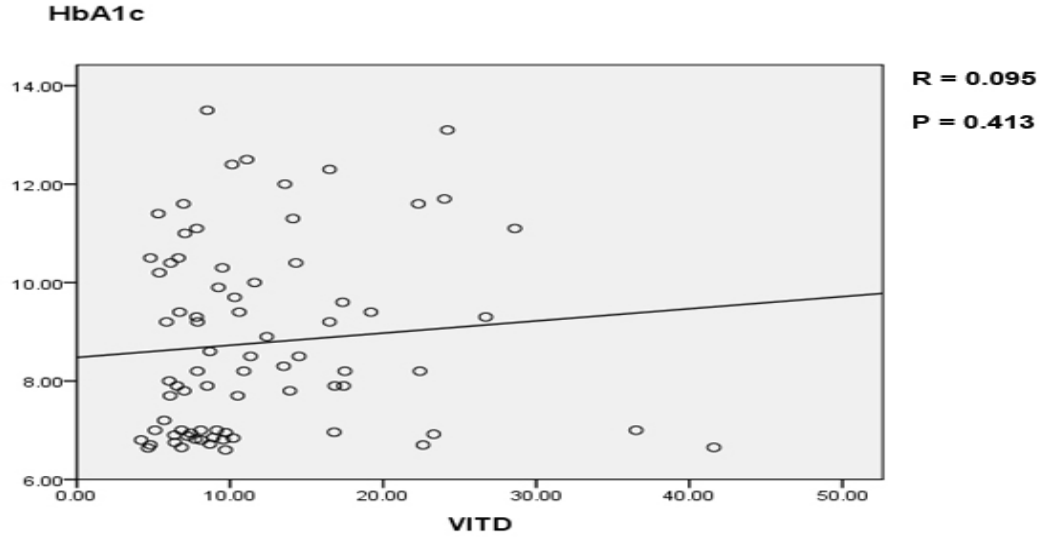


Fig 6: Correlation between vit.D and CRP in obese group

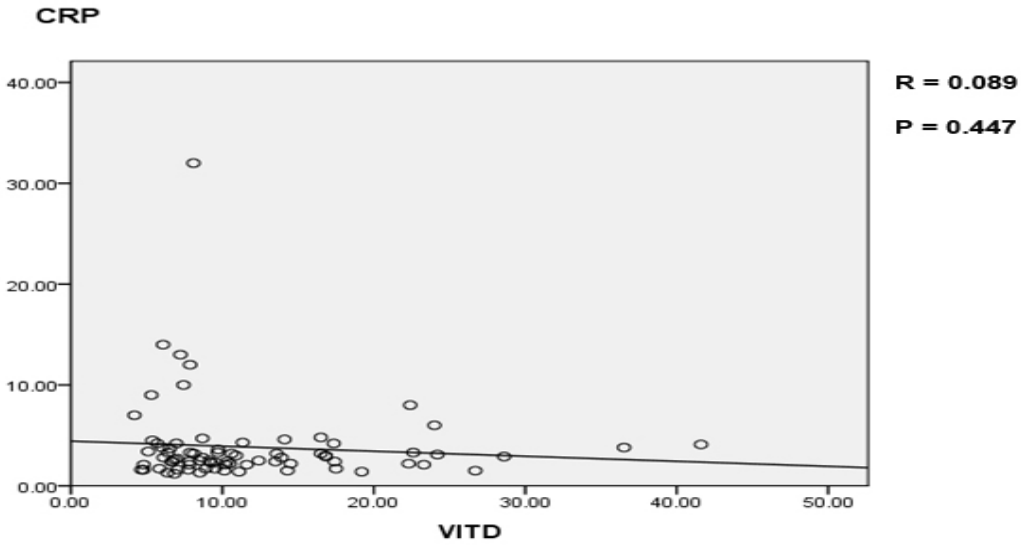
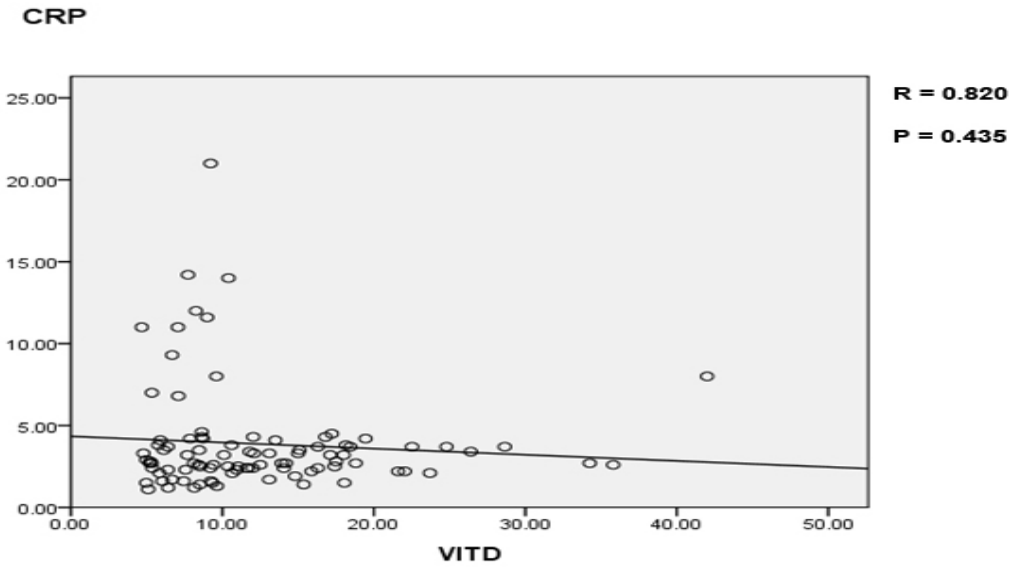


Fig 7: Correlation between vit.D and CRP indiabetic group



Discussion:

Vitamin D deficiency is considered a public health problem around the world. In 2008, it was estimated that 1 billion individuals presented vitamin D insufficiency or deficiency[19]. Serum concentration of 25(OH) VIT.D is the best indicator of vitamin D status. It reflects vitamin D produced cutaneously and that obtained from food and supplements[20] and has a fairly long circulating half-life of 15 days[21]. 25(OH) vit.D functions as a biomarker of exposure, but it is not clear to what extent 25(OH) vit.D levels also serve as a biomarker of effect[20]. Serum 25 (OH) vit.D levels do not indicate the amount of vitamin D stored in body tissues. In contrast to 25(OH) vit.D, circulating 1, 25 $(OH)_2$ D is generally not a good indicator of vitamin D status because it has a short half-life of 15 hours and serum concentrations are closely regulated by parathyroid hormone, calcium, and phosphate[1]. Levels of 1, 25$(OH)_2$

vit.D do not typically decrease until vitamin D deficiency is severe[1,22]. In an international study of women with osteoporosis, the highest prevalence of hypovitaminosis D was reported in the Middle East[23]. Indeed, developed Arab Gulf countries carry a high prevalence of hypovitaminosis D, a finding possibly explained by cultural practices and skin pigmentation[24] which is supported by our study that showed Prevalence of vitamin D deficiency was very higher among studied groups.

Type 2 DM represents a significant global health problem. It is estimated that about six people die every minute from the disease worldwide, a figure that will soon make type 2 DM one of the world's most prevalent health problems[25]. Basic science studies revealing the pleiotropic effects of vitamin D on calcium metabolism, insulin secretion and insulin action as well as being an easily administered agent, have made vitamin D an ideal candidate for modulating the development of the metabolic syndrome and type 2 DM [11]. There are some conflicting data from human studies that have directly examined the relationship between vitamin D or calcium status and systemic inflammation in relation to type 2 DM[26]. However, the association remains inconsistent and this may reflect confounding factors, such as genetic variation affecting the individual responses to vitamin D[27]. We found that despite a common finding of vitamin D deficiency, hypovitaminosis D is not associated with increased prevalence of type 2DM and obesity in both patients' groups. Neither is there any relationship between vitamin D levels and glycaemic control (FBG& HbA1c) or cellular inflammation in all groups which is supported by many studies; some intervention studies have shown inconclusive results on the effect of vitamin D on HbA1c and type 2 DM [28] and also in the Women's Health Initiative, a cohort of 33 951 postmenopausal women were randomized to receive calcium 1 g and vitamin D3 400 IU, or placebo.68 Over a 7 year follow-up, supplementation did not appear to reduce the risk of developing type2 DM[10]. In a cross-sectional survey carried out in New Zealand on 5677 subjects aged 40-64 years, serum concentrations of 25(OH)D3 were significantly lower in newly detected cases with diabetes and IGT (n = 238) compared to their healthy age- and sex-matched controls. The authors concluded that low serum 25(OH) levels might somehow predispose to IGT and type2 DM [29]. This conclusion could not be justified as both diabetics and their healthy counterparts had sufficient circulating 25(OH) D (69 ± 31 vs. 76 ± 34 nmol/L) respectively. In another pilot study the prevalence of vitamin D insufficiency was reported higher in type 2DM than in type1 DM[30]. However, neither the patients nor the healthy subjects were vitamin D deficient (82.5±1.3 vs. 96.7±2.0 nmol/L, respectively). It is therefore unlikely that "lowered" serum levels of25(OH) vit.D, while still far above desirable level, could induce auto-immunity[31]. High prevalence of vitamin D insufficiency in the studied groups weakens the possibility of finding any significant difference in the circulating 25(OH) D between healthy and diabetic subjects. While others reported that Vitamin D levels negatively correlate with BMI and fat mass [32] and obesity alters the release of vitamin D into the circulation and decreases its bioavailability because of deposition in adipose tissue compartments[33].

Our study supported the decreased bioavailability of serum vitamin D observed in obese individuals from previous studies[34,35]. Wortsman et al.[35] found obesity did not affect the skin's ability to produce vitamin D, but may have altered the release of vitamin D into the circulation from adipose stores due to increased subcutaneous fat. As a fat soluble molecule, decreased 25(OH) vit.D levels among obese individuals may be due to enhanced uptake in adipose tissue and metabolic clearance[34]. We measured parathyroid hormone, a strong point in our study, since hyperparathyroidism; secondary to hypovitaminosis D is augmented by obesity. We did not find any effect of apparently hypovitaminosis D on parathyroid hormone level in obese group. The inverse relationship between 25(OH) vitamin D and parathyroid hormone (PTH) levels is well established, and PTH in part regulates production of 1-25$(OH)_2$ vitamin D. It has been reported that elevated 1-25$(OH)_2$ vitamin D stimulates lipogenesis and triglyceride accumulation and inhibits lipolysis[36]. Parikh et al.[37] who studied relationship between obesity and active vit.D in a cross-sectional, observational study and found that

1-25$(OH)_2$ vitamin D levels are elevated in people with a BMI ≥ 30 kg/m2, but these levels fall as obesity increases, making it unlikely that elevated 1-25(OH) vitamin D levels are lipogenic. Despite these associations, vitamin D deficiency has not been clearly identified as the cause or the outcome of obesity. Nevertheless, the question can still be raised that whether this extensive vitamin D deficiency in our population has some role in the increasing occurrence of type 2 DM and obesity. So in our study we measured the iPTH in all studied groups aiming to find any possible relation with hypovitaminosis D and unfortunately there was not any significant relations between 25(OH) vit.D & iPTH in diabetic group which can be explained by the possibility that 25(OH) vit.D did not relate to and was not a good indicator for active vit.D (1, 25$(OH)_2$vit.D. it was suggested that several genetic polymorphisms in genes related to vitamin D metabolism, such as DBP and VDR, may predispose subjects to type 2 DM[38]. VDR polymorphisms were also associated with type 2 diabetes in two Indian case-control studies.[39, 40] Recently, the role of common genetic variants on vitamin D status has begun to be elucidated.[41,42] Therefore, genes along the vitamin D metabolic pathway and action and those along the innate immunity-related inflammation can interact to influence the preventive efficacy of vitamin D on type 2DM. Employing such a multiplexed approach in relation to vitamin D and inflammation would allow for assessing its effects on multiple biomarkers simultaneously. This assessment may also permit to develop an "omics"-based signature to distinguish responders to vitamin D intake from non-responders to whom alternative preventive measures can be introduced.[27] Well-designed clinical trials of the effect of vitamin D supplementation on glycaemic status and diabetes risk are urgently required to answer the question. And they need to prevent past mistakes. In particular, the vitamin D dose given in such trials needs to be high enough-above 2,000 IU per day[43]- to raise blood 25(OH)D levels above 80 nmol/l because diabetes risk is lowest at this level [44]. If well-designed trials are carried out and confirm a protective effect from vitamin D, it could be used by the general population as a simple and cheap solution to help prevent the diabetes epidemic.

In summary, there is no relationship between hypovitaminosis D and obesity or glycaemic control in well-established type 2 DM. Future studies specifically designed to investigate the role of vitamin D on type 2 diabetes using inflammation as the main outcome are urgently needed in order to provide a more forceful link between vitamin D, inflammation and type 2 DM. Furthermore, genetic polymorphisms studies are also important in order to identify groups that are more susceptible to vitamin D deficiency and to developing type2 diabetes in the population.

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Original Article

Relation of Insulin Resistance and Hepatocellular Carcinoma in Non-Obese Non-Diabetic Hepatitis-C Virus Positive Patients.

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Abstract:

Background: The higher incidence of insulin resistance (IR) in patients with hepatitis-C virus (HCV) infection is becoming an increasing problem. IR has emerged as a risk factor for a wide variety of cancers. It has been reported that IR can increase the risk of developing Hepatocellular carcinoma (HCC) in patients with chronic HCV infection. The aim of the present study is to assess the relationship between insulin resistance and HCC in non-obese non-diabetic HCV positive Egyptian patients **Patients & methods:** The study included 60 chronic HCV positive patients divided into two groups: Group I consist of 30 HCV+ve patients with HCC and group II consists of 30 HCV+ve patients without HCC. Ten healthy individuals are selected as a control group. Full history taking and clinical examination were done for all patients and controls. Body mass index (BMI) was recorded. A blood sample was withdrawn for complete blood count (CBC), aspartate transaminase (AST), alanine transaminase (ALT), albumin, bilirubin, prothrombin activity (PT%), insulin, fasting blood glucose (FBG), 2-hour post load blood glucose, urea, creatinine and alpha fetoprotein. Homeostasis-model assessment of insulin resistance (HOMA-IR) was calculated. Diagnosis was done clinically, by abdominal ultrasonography and by assessing viral markers (HBsAg, HCV Ab and quantitative PCR for HCV- RNA). HCC is further confirmed by triphasic CT of the liver and AFP.(Alpha fetoprotein) **Results:** There was no significant difference with regard to gender, BMI, FBG and 2 hour post load blood glucose (P>0.05) among studied groups. There was a significant difference among studied groups with regard age, platelet count, ALT, AST, serum albumin, PT%, total bilirubin, serum insulin, HOMA-IR and AFP levels (P<0.05). Group I was significantly older than group II (P=0.03) and controls (P=0.04). Platelets count and serum albumin level of group I were significantly lower than group II (P=0.009 and 0.001 respectively). Platelets count, serum albumin level and PT% of group I were significantly lower than controls (P=0.002, 0.001 and 0.01 respectively). Total bilirubin, AST and ALT levels of group I were significantly higher than group II and controls (P=0.001 for all). Serum insulin level of group I were significantly higher than group II and controls (P=0.04 and 0.001 respectively). HOMA-IR level of group I was significantly higher than group II and controls (P=0.001 for both). Serum insulin and HOMA-IR levels of group II were significantly higher than controls (P=0.03 for both). Multivariate logistic regression analysis showed that HOMA-IR and insulin level were independent predictors for the risk of HCC development (P=0.04 and 0.03 respectively). **Conclusion:** This study showed a significantly higher degree of insulin resistance in patients with HCV infection and HCC compared with HCV infection alone or healthy controls. We hypothesize that the presence of a vicious cycle triggered by HCV infection leads to increased insulin resistance with subsequent increased risk of HCC.

Key Words: HCC, HCV, insulin resistance, HOMA-IR.

Introduction:

Infection with hepatitis C virus (HCV) is a common problem worldwide, affecting millions of people across all populations. Most acutely infected patients develop chronic hepatitis and become a potential source of virus transmission, and as many as one patient in every five patients will develop cirrhosis and its complications such as Hepatocellular carcinoma (HCC).[1] HCC is the commonest primary cancer of the liver. Incidence is increasing and HCC has risen to become the 5th commonest malignancy worldwide and the third leading cause of cancer related death.[2] The major known risk factors for HCC are viral (chronic hepatitis B and hepatitis C), toxic (alcohol and Aflatoxin) and immune-related

primary biliary cirrhosis and autoimmune hepatitis.[3]

Understanding the risk factors for HCC development in patients infected with HCV is thus of great importance for refinement of treatment strategies and healthcare delivery. Egypt has the highest countrywide prevalence of HCV in the world; about 12 to 15% of the total population is infected.[4]

The higher incidence of insulin resistance and DM in patients with HCV infection is becoming an increasing problem. Moreover, the association of HCV infection and DM is present, even before the onset of cirrhosis.[5] Diabetes was associated with a two - to threefold increase in HCC risk regardless of chronic HCV or HBV infection or alcoholic liver disease. Also, increased risk of liver cancer fourfold was reported among patients with diabetes in the presence of hepatitis, cirrhosis, and alcoholism.[6] Metabolic syndrome has been implicated as a risk factor for non-alcoholic fatty liver disease (NAFLD), including in its most severe form, non-alcoholic steatohepatitis (NASH). NASH has been identified as a cause of both "cryptogenic cirrhosis" and HCC.[7] Insulin resistance **(**IR) is a key feature of this syndrome and a variety of potential molecular pathways by which HCV may contribute to IR have been suggested.[8,9] IR is a complex pathophysiological condition where higher-than-normal concentrations of insulin are needed to maintain a normal glycemia and adequate glucose utilization in insulin target tissues.[10] IR is extremely common in patients with chronic HCV infection and has been associated with increased disease severity, fibrosis progression, hepatic steatosis, extrahepatic manifestations and decreased response to antiviral therapy.[11, 12]

IR has emerged as a risk factor for a wide variety of cancers, including endometrial, breast, colon, rectal, esophageal, kidney, pancreatic, biliary, ovarian and cervical cancers.[13] It has been reported that IR can increase the risk of developing HCC in patients with chronic HCV infection.[14]

Since HCV is endemic in Egypt and IR is a potentially modifiable factor, a better understanding of the association of IR with HCC among Egyptian patients should be considered.

The aim of the present study is to assess the relationship between insulin resistance and hepatocellular carcinoma in non-obese non-diabetic HCV positive Egyptian patients.

Patients and Methods:

The study included 60 chronic HCV positive patients (previously diagnosed by PCR for HCV-RNA) selected from internal medicine and tropical medicine departments in menofiya university hospital. The patients are divided into two groups: Group I consists of 30 HCV positive patients with HCC and group II consists of 30 HCV positive patients without HCC. They are 50 males and 10 females ranged between 35-76 years old. Ten healthy individuals are selected as a control group (Group III). All patients and controls gave their written informed consent before participating in the study.

Exclusion criteria included HBsAg positive patients, patients with concomitant causes of chronic liver diseases and history of alcohol consumption, diabetic patients and patients with BMI more than 30 kg/m^2.

Full history taking and complete clinical examination were done for all patients and controls. Body mass index (BMI) was recorded. A blood sample was withdrawn by sterile venipuncture and divided into three parts: EDTA was added to the first sample which was used for complete blood count (in cell counter, Pentra 80, France). The second sample was put in plain vacutainer tube which was left to clot at 37ºC, centrifuged in a plain container. Serum was used to estimate AST, ALT, albumin, bilirubin (on Synchron CX5 autoanalyser). Also, Insulin level, blood urea, serum creatinine, FBG, 2 hour post load blood glucose and AFP were estimated. Third sample with sodium citrate was used to estimate prothrombin activity. The index of insulin resistance was calculated on the basis of fasting values of plasma glucose and insulin, according to the homeostasis model assessment (HOMA) method, HOMA - IR = (glucose in mmol/L × insulin in µIu/mL/22.5).

Diagnosis was done clinically, by abdominal ultrasonography (liver and spleen

status and degree of ascites) and by assessing viral markers (HBsAg, HCV Ab and quantitative PCR for HCV- RNA). HCC is further confirmed by triphasic CT of the liver and AFP.

Statistical Analysis:

Results are presented as mean ± standard deviation (SD) unless otherwise stated. For comparison of two means, the unpaired t test and non-parametric Mann-Whitney test were used. The ANOVA test with post hoc was used to compare among HCV positive patients with and without HCC and controls. Fisher Exact analysis was also applied to compare proportions between groups. Multiple logistic regression analysis was used to detect the independent risk factors of HCC. A p of ≤ 0.05 was considered statistically significant. All statistical analyses were performed using the Statistical Package for Social Science (SPSS) software version 10.

Results:

The study included 60 chronic HCV positive patients. The patients were divided into two groups. Group I consists of 30 HCV positive patients with HCC. They are 25 male and 5 female and their age is ranged between 45 and 76 years. Group II consists of 30 HCV positive patients without HCC. They are 25 male and 5 female and their age is ranged between 35 and 63 years. Group III consists of ten healthy individuals (control group) 7 males and 3 female their age ranged between 30 and 55 years.

Comparison among HCV positive patients with HCC (group I), HCV positive patients without HCC (group II) and controls (table 1 and 2) showed that there was no significant difference with regard to gender, BMI, Hb level, serum creatinine, FBG and 2-hour post load blood glucose (P>0.05). There was a significant difference among HCV positive patients with HCC (group I) and without HCC (group II) and controls with regard age, WBC, platelet count, blood urea, ALT, AST, serum albumin, PT%, total bilirubin, serum insulin, HOMA-IR and AFP levels (P<0.05). HCV +ve patients with HCC were significantly older than HCV +ve patients without HCC (P = 0.03) and controls (P = 0.04) but there was no significant difference between age of HCV positive patients without HCC (group II) and controls (P value=0.08). Platelets count and serum albumin level of HCV positive patients with HCC were significantly lower than HCV positive patients without HCC (P= 0.009 and 0.001 respectively). Platelets count, serum albumin level and PT% of HCV positive patients with HCC were significantly lower than controls (P= 0.002, 0.001 and 0.01 respectively). Platelets count, serum albumin level and PT% of HCV positive patients without HCC was significantly lower than controls (P= 0.01, 0.03 and 0.001 respectively). Total bilirubin, AST and ALT levels of HCV positive patients with HCC were significantly higher than HCV positive patients without HCC and controls (P= 0.001 for all). Total bilirubin, AST and ALT levels of HCV positive patients without HCC were significantly higher than controls (P= 0.03, 0.002 and 0.001 respectively). Serum insulin level of HCV positive patients with HCC were significantly higher than HCV positive patients without HCC and controls (P= 0.04 and 0.001 respectively). HOMA-IR level of HCV positive patients with HCC were significantly higher than HCV positive patients without HCC and controls (P= 0.001 for both). Serum insulin and HOMA-IR levels of HCV positive patients without HCC were significantly higher than controls (P= 0.03 for both). Blood urea level of HCV positive patients with HCC was significantly higher than HCV positive patients without HCC and controls (P= 0.001 for both). Blood urea level of HCV positive patients without HCC was significantly higher than controls (P= 0.001).

The severity of the liver disease was graded according to Child-Pugh's criteria. Eight patients (26.6%) belonged to Child's class A in group II. Twenty one patients (70%) belonged to Child's class B in group I and 18 patients (60%) in group II. Nine patients (30%) belonged to Child's class C in group I and 4 (13.3%) in group II. Patients with HCC had significantly higher Child-Pugh score than

HCV positive patients without HCC (9.3±1.7 versus 7.4±1.1) (P= 0.03).

Ascites is more frequent in HCV positive patients with HCC (87%) than HCV positive patients without HCC (30%) (P =0.003).

Encephalopathy is more frequent in HCV positive patient with HCC (26.7%) than HCV positive patient without HCC (6.7%) but it did not reach significant value (P=0.08).

Multivariate logistic regression analysis (Table 3) showed that HOMA-IR and insulin level were independent predictors for the risk of HCC development (P=0.04 and 0.03 respectively).

Table 1: Comparison among HCV positive patients with HCC (group I), HCV positive patients without HCC (group II) and controls:

	Group I (n = 30)	**Group II (n = 30)**	**Controls (n = 10)**	**P-value**
Age (years)	61.7±9.5	46.3±10.2	38.9±4.7	0.04*
Gender (Male %)	83.3% (n=25)	83.3% (n=25)	70% (n=7)	0.7
BMI (kg/m^2)	27.4± 1.5	26.5 ±1.3	23.9 ±1.2	0.37
Hb (gm/dl)	9.9±0.7	11.7±1.1	12.7±0.8	0.09
WBC ×10^3 (U/L)	8.2±4.4	8.7±2.1	7.4±1.5	0.03*
Platelets ×10^3 (U/L)	68.1± 7.6	132.8± 1.6	395± 1.9	0.008*
Urea (mg/dl)	69.6±26.6	38.1±16.5	14.7±5	0.001*
Creatinine (mg/dl)	1.68± 0.77	1.54± 0.65	1± 0.15	0.25
FBG (mg/dl)	95.5 ± 10	91.6 ± 9	80.8 ± 6.4	0.06
2-hour post load blood glucose (mg/dl)	130.3 ± 4.6	131.6 ± 3.8	127 ± 5.9	0.32
Insulin (µlu/ml)	14.8 ± 4.55	5.9 ± 3.2	3 ± 1.6	0.02*
HOMA-IR	3.43 ± 1.1	2.1 ± 0.7	0.58 ± 0.31	0.001*
AFP (ug/dl)	679.6±88	5.8±4.38	2.7±1.88	0.001*

BMI= Body mass index, **Hb=** Hemoglobin, **WBC=** White blood count, **FBG=** Fasting blood glucose, **AFP=** Alfa fetoprotein, **n =** number of patients

HOMA-IR= Homeostasis model assessment of insulin resistance

Table 2: Liver function tests among HCV positive patients with HCC (group 1), HCV positive patients without HCC (group II) and controls:

	Group 1 (n=30)	**Group II (n = 30)**	**Controls (n = 10)**	**P-value**
ALT (U/L)	104.6±13	72.6±4.5	13.6±1.3	0.04*
AST (U/L)	82.3±9.4	51.3±4.2	16.9±2	0.003*
Albumin (gm/dl)	2.76±0.37	3.27±0.63	4.55±0.37	0.001*
Total bilirubin (mg/dl)	2.83±1.3	1.78±0.28	0.55±0.15	0.01*
PT%	51.1±7	56.7±5.96	95.9±2.68	0.01*

ALT=Alanine transaminase **AST**= Aspartate transaminase **PT%**=Prothrombin activity

Table3. Multivariate regression analysis for risk factors of HCC in HCV+ve patients

Risk factors	P-value	R
Age (years)	0.5	0.02
Serum insulin (µlu/ml)	0.03*	0.36
Albumin (gm/dl)	0.6	0.01
HOMA-IR	0.04*	0.34
AST (U/L)	0.7	0.01
ALT (U/L)	0.8	0.01
Total bilirubin (mg/dl)	0.3	0.01

HOMA-IR= Homeostasis model assessment of insulin resistance
AST= Aspartate transaminase, **ALT=** Alanine transamine

Discussion:

HCC is one of the commonest cancers worldwide. It is a major health problem and its incidence is increasing. The cause of this increase in HCC is incompletely understood. The presence of cirrhosis is the major risk factor and this is largely due to chronic HCV and HBV infection. HCC carcinogenesis is likely to involve interplay of viral, environmental and host factors.[2] In general, approximately 15–50% of HCC cases remain idiopathic, suggesting that other risk factors are responsible for this increase in HCC.[15]

Diabetes has been suggested as a potential risk factor for HCC in patients with chronic hepatitis C.[16] Development of diabetes-related HCC is reported to be independent of viral hepatitis and alcoholism.[17] Moreover, diabetes is a risk factor for non-alcoholic liver disease (NAFLD), including its most severe form, non-alcoholic steatohepatitis (NASH), which can lead to cirrhosis and subsequently HCC.[7]

Insulin resistance is frequently seen in patients with hepatitis HCV infection.[18] Harrison and co-workers[19] reported that 30 to 70% of chronic HCV patients display some evidence of IR. These results have suggested the occurrence of IR at early stage(s) of chronic HCV infection irrespective of the severity of liver disease and thus the possible role of IR as a metabolic factor that increases risk of HCC development.[20]

Although in the general population, lack of exercise and overeating are major causes of insulin resistance, in patients with HCV infection, hepatic inflammation, activated inflammatory cytokines, and HCV-induced impairments of insulin and lipid signaling molecules are also important factors for the development of insulin resistance.[21] Intracellular fat accumulation by itself causes insulin resistance.[22] Experimental interventions that increase hepatic triglyceride content in mice result in defects in insulin signaling and impairment of the ability of insulin to suppress hepatic glucose production.[23] Alterations in hepatic lipid and carbohydrate metabolism are commonly observed in chronic hepatitis C and it was noted that hepatitis C was associated with hepatic steatosis to a greater extent than other inflammatory liver diseases.[24] Moreover, hepatic steatosis is considered as an independent risk factor for disease progression in patients with HCV infection.[25] Therefore, Insulin resistance is closely associated with progression of hepatic fibrosis in patients with HCV infection.[26] Insulin resistance may directly affect hepatic stellate cells and increase connective tissue growth factor, which causes production of extracellular matrix.[27] Alternatively, insulin resistance-induced hepatic lipid accumulation may increase oxidative stress, resulting in progression of hepatic fibrosis.[28]

The present study showed a significantly higher degree of insulin resistance (as indicated by the raised HOMA index) in patients with

hepatitis C infection compared to the control group. This finding supports a role for HCV in the development of insulin resistance and DM as previously reported.[6, 29]

Generally, insulin resistance results in the development of type 2 diabetes mellitus and increases the risk of life threatening complications such as cardiovascular diseases, renal failure, and infections.[30] However, these complications are not major causes of death in cirrhotic patients with insulin resistance. On the other hand, the development of intrahepatic complications, including HCC, is associated with insulin resistance.[12] Insulin resistance is also reported to be involved in the development of extrahepatic manifestations of HCV infection including gastric cancer and is associated with a poor response to anti-viral treatment.[21,31]

These findings suggest that insulin resistance has direct effects on hepatocarcinogenesis. Insulin resistance causes lipid accumulation.[32] Visceral adiposity results in changes in serum adipocytokine levels, including reduction of adiponectin, which has suppressor effects for hepatocarcinogenesis.[33] Hepatic lipid accumulation also increases oxidative stress, which may be responsible for the development of HCC.[34] Beside these possibilities, insulin has a mitogenic effect, suggesting that insulin may be directly linked to hepatocarcinogenesis.[32] In turn, IR and compensatory hyperinsulinaemia can promote the synthesis and biological activity of insulin – like growth factor 1(IGF-1), which stimulates cell proliferation and inhibits apoptosis, and has been shown to have a strong mitogenic effects on a wide variety of cancer cell lines, including HCC.[20] Excess insulin might also affect the development of cancer indirectly by downregulating the level of IGF-binding protein1, which increases the level and bioavailability of total circulating IGF-1.[35]

Insulin resistance may be associated not only with hepatocarcinogenesis, but also with proliferation of HCC. Insulin exerts growth-promoting activity through activation of a mitogen-activated protein kinase pathway.[32] In addition, overexpression of transducing molecules for insulin signaling, IRS1 and IRS2 occur in HCC.[36,37] Thus, HCC may be sensitive to insulin stimulation.

In diabetic patients, treatment with sulfonylurea or exogenous insulin increases HCC risk and worsens tumor response to radioablation, whereas HCC patients taking the insulin-sensitizing drug metformin have more favorable outcomes.[38] This finding suggests a direct role for insulin in liver tumor promotion. Previous studies suggest a strong synergistic effect of metabolic factors and viral hepatitis in HCC development in HCV-infected patients.[6, 39]

In the present study, HOMA-IR was significantly higher in patients with HCV infection and HCC than HCV positive patients without HCC and controls. Moreover, serum insulin level was significantly higher in patients with HCV infection and HCC compared to patients HCV infection alone or the control group. This work elucidated an independent association between HOMA-IR and development of HCC regardless diabetes and obesity as previously described.[14,40] In contrast, Mohamed et al showed no significant difference in HOMA-IR values, diabetes and insulin levels between HCV positive patients with and without HCC.[41]

Reduction of fasting blood glucose and hemoglobin A1c is a well-established therapeutic strategy for prevention of complications in diabetic patients.[30] However, therapeutic guidelines for inhibiting the distinctive complications of HCV-associated insulin resistance are not yet available. Moreover, amelioration of insulin resistance is considered to inhibit complications and improve prognosis.

Conclusion:

This study showed a significantly higher degree of insulin resistance in patients with HCV infection and HCC compared with HCV infection alone or healthy controls. We hypothesize that the presence of a vicious cycle triggered by HCV infection leads to increased insulin resistance with subsequent increased risk of HCC. Moreover, this study complements studies of HCV infection in other populations and indicates that in the Egyptian population suffering from a high burden of hepatitis C, the strikingly high rates of hepatocarcinogenesis result from a combination of direct viral effect and the influence of an array of metabolic factors resulting from virus-induced insulin resistance. A better

understanding of IR involvement in HCV mediated HCC may point to potential for benefit of therapeutic interventions aimed at control of IR, in these patients.

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Original Article

Impact of Quality Control Measures in Prevention of HCV Seroconversion in Haemodialysis Patients.

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Abstract:

Background: HCV infection is very prevalent among the haemodialysis (HD) patients and constitutes a major cause of morbidity and mortality among these patients. This study is designed to reduce the HCV seroconversion rate among haemodialysis patients. **Methods:** A total number of 382 HD patients in King Fahad Kidney center (KFKC) were recruited in the study in a period of 2 years, from January, 2012 to December, 2013. The quality control measures were implemented effectively in March, 2013. HCV seroconversion rates were assessed every 3 months during the observation period. **Results:** The prevalence of HCV cases in the beginning of the study was 23.3%. After 1 year the prevalence of HCV cases increased to be 24.3% and HCV seroconversion rate was 1% by the end of the 1st year. Outbreak of HCV infection occurred in the following 3 months and the prevalence increased to 25.1% and seroconversion rate recorded 0.78% in these 3 months only. After effective application of the quality control measures, no further HCV seroconversion were recorded in the following 9 months till the end of the study and the seroconversion rate was 0% (significant reduction, $\chi^2 = 8.3$,P = 0.003). **Conclusion:** Effective implementation of the quality control measures on the haemodialysis patients decreased the seroconversion rates of HCV as a blood borne disease among these patients.

Key words: quality control measures, haemodiaysis, hepatitis C virus.

Introduction:

Hepatitis C virus (HCV) infection represents a big public health challenge in this millennium and the World Health Organization estimates that, until 1998, 170 million people carried the HCV worldwide and in recent years 200 million or 3% of the world population is infected with this virus[(1)].

The prevalence of HCV infection in patients undergoing dialysis is greater than that in the general population, suggesting that patients on dialysis may be at higher risk of acquiring HCV infection. This is predominantly because these patients are more exposed to risk factors for the acquisition of this infection and also because they are monitored monthly by laboratory examinations that permit an early diagnosis of the infection[(2)]. The prevalence of HCV infection in hemodialysis patients(HD) can vary from 7% up to 70% in some countries[(3,4)].

In contrast with the hepatitis B virus (HBV), no vaccine is available for HCV[(7)]. Accurate testing for HCV is complicated by regional variation in the HCV genome and by variation in screening tests[(4-8)]. Patients infected with HCV often have minimal clinical evidence of disease[(6,7,8)]. HCV infection in end-stage renal disease (ESRD) patients has been associated with greater morbidity and mortality[(6,10,11)].

There are many quality control measures to minimize the transmission of blood-borne infections including HCV in patients with ESRD already on HD.

These measures include hand hygiene protocols, awareness programs, aseptic technique policies, using single dose vials of heparin instead of multiple dose vials, reduction of the use of the central line access, environmental planning, staff planning, controlling of HD center entrances, improving bed and machine conditions and using erythropoietin to reduce blood transfusion[(12-15)].

Chronic hepatitis C virus infection is found with variable prevalence in dialysis populations in different parts of the world. Outbreaks of hepatitis C has been reported in King Fahad Kidney Center (KFKC) in Riyadh,

KSA. In 2012 four patients were seroconverted from anti-hepatitis C virus (HCV) antibodies negative to anti-HCV antibodies positive. On the other hand, in first quarter of 2013, the Prevention and Control of Infection Department reported three new cases were seroconverted to HCV positive among hemodialysis patients of the center.

This project is designed to prevent seroconversion of HCV in dialysis patients.

Patients & Methods:

A total number of 382 patients with ESRD already on HD at least for 6 months were followed up in KFKC for 2 years; from January 2012 to December 2013. Application of the standard quality control program measures for HD patients was effective starting March, 2013. Patients were checked for HCV antibodies & PCR, HBV screen also was performed. Patients who were reactive to HBV were excluded from the study. All other causes of liver dysfunction & treatment with interferon &/or ribavirin were excluded. A case of HCV was defined as a patient diagnosed before as having HCV or was HCV antibody-positive at the time of entry of the study. Serology for HCV was done for our studied patients every 3 months. HCV seroconversion was defined as any patient was HCV antibody negative at the entry of the study then became positive at any time during the study.

Quality Control Measures Of HD Patients:

The quality control measures of HD in KFKC includes the following items:

Awareness programs: Lecture hall policies and procedures & PPT (power point) presentation.

Demonstrate proper aseptic techniques**:** Aseptic techniques policy, education sessions, audit sheet.

Demonstrate proper hand hygiene program: Hand hygiene policy, education sessions, audit sheet. Monitor staff performance regarding the aseptic techniques practice through conducting audit round by the multidisciplinary team. Conduct Hand Hygiene Campaign. Provision of hand out material to increase staff compliance to hand hygiene practice. Increase hand rub and hand washing stations.

Using Heparin single dose vial policy instead of multiple doses.

Reduction of using the central line access & provision of pre and post dialysis disposable procedure sets.

Improving the environmental cleaning: Conduct awareness sessions to patient about caring their central line access. Monitor nursing staff performance regarding the cleaning of particularly HD beds and machines and house keeper for cleaning the floors and walls. Update director of the HD center and director nursing to pre and post cleaning data sheet in regular bases. Train nursing staff and house keeper on the correct practice of HD machine disinfection and use the cleaning material. Increase distance between HD beds to be 1.5 meters.

Nursing manpower improvement plans: application of 2:1 patient –staff nurse ratio. Decrease shortage of staff.

Controlling the HD center entrances through security department improvement: Secured all KFKC facilities &increase security men.

Improving bed sharing & machine type for every patient: Standardized bed number and machine type to every patient, standardized fixed duty of nurses to patients on weekly bases.

Medical supply improvement: as changing the old HD machines by new version ones.

The quality control center in KFKC did a regular & continuous supervision & check up for all the parameters and the percentage of achievement of the targets of the quality control programs in HD units of KFKC.

A regular reporting to the quality control center from each HD unit including database & the cases of HCV including the seroconversion cases.

Statistics & Statistical Methods

The main item of our study was HCV cases as regards to the prevalence and seroconversion rates. The prevalence of HCV cases was studied as a cross sectional study of HCV cases among our HD patients. Seroconversion rates were checked every 3 months and were defined as any case with HCV antibodies negative at the entry of the study and changed at any time during the study into HCV antibodies positive. The seroconversion rates were represented as the number of the seroconversion cases per 100 patients every 3months. Chi- square test was used to compare between the different groups. P was considered statistically significant if < 0.05. SPSS software program (SPSS Inc., USA) was used.

Results:

The baseline clinical &demographic data of the studied patients are present in table 1. The number of patients was 382, 210 (55%) were males & 172 (45%) were females. The mea age of the patients was 55.6 years ±13.7 years. The mean duration of HD was 33.2±29.6 months. The numbers of each group of patients according to the etiology were; diabetic nephropathy: 152 (39.79%), hypertension nephropathy: 127(33.24%), primary glomerular disease: 29(7.59%), obstructive uropathy: 8 (2.1%), unknown disease: 33(8.63%), other causes: 33 (8.66%).

The duration of the study was 2 years (from January, 2012 to December, 2013). The number of HCV cases in our HD patients at the beginning of our study in January, 2012 was 89 (23.3%) patients, 4 cases were seroconverted by the end of 2012, so HCV cases were 93 (24.3%)&the seroconversion rate in 2012 was 1%. Outbreak of seroconversion of additional 3 cases were added in the first quarter in 2013. HCV cases were 96 (25.1%). The seroconversion rate in these 3 months only was 0.78%. After effective application of the HD quality control in March, 2013, no seroconverted cases were recorded till the end of the study in December, 2013. So, the seroconversion rate to HCV was 0% after effective application of the quality control measures. These results showed significant reduction of the HCV seroconversion rate during the study (χ^2 = 8.3, P = 0.003).

Quality Control Measures:

During the observation period, the attendance rate of the awareness programs was 92% & 80% of the staff were compliant to aseptic technique policy & 60 procedures were monitored per month. 70% of the staff were compliant to hand hygiene policy practice. There was 55% reduction in the rate of using central line in HD in 2013 & 90% of the KFKC staff attended the education sessions. Reduction of the shortage of the nursing staff to 10% by supporting 36 new staff. Adding 40 new HD beds & 78 new version of HD machines.

HCV Seroconversion Rates:

Outbreaks of hepatitis C has been reported in King Fahad Kidney Center. In 2012, 4 patients are seroconverting from anti-hepatitis C virus (HCV) negative to anti-HCV positive (the seroconversion rate was 1%) & 3 additional patients were seroconverted in the first quarter of 2013 among hemodialysis patients (the seroconversion rate in these 3 months only is very high ; 0.78%) . However application of the infection control program was done in March, 2013, the last 9 months of the observation study no cases of HCV seroconversion were recorded till the end of the study (in December, 2013.

Table1: the basic demographic & clinical data of the studied patients

Variable item	Number
Total number of patients	382
Male (no, %)	210 (55%)
Female (no, %)	172 (45%)
Duration of the study (in years)	2
Duration of HD (in months)	33.2 ± 29.6
Age (no ± SD) years	55.6 ±13.7
Hypertension nephropathy (no,%)	127 (33.24 %)
Diabetic nephropathy (no,%)	152 (39.79 %)
Primary glomerulopathy (no,%)	29 (7.59%)
Idiopathic KD (no,%)	33 (8.63%)
Obstructive uropathy (no,%)	8 (2.09 %)
Other causes (no,%)	33 (8.66%).
HCV patients at the beginning of the study in Jan 2012 (no,%)	89 (23.3%)
HCV patients at the end of 2012 (no,%)	93 (24.3%)
No & % of seroconversion cases in 2012	4 (1%)
HCV patients at the end of March, 2013 (no& %)	96 (25.1%)
Seroconversion cases &rate from Jan to Mar,2013	3 (0.78%)
HCV cases by the end of 2013	96 (25.1%)
Seroconversion rate(from Mar to end 2013)(%)	0%

Figure 1

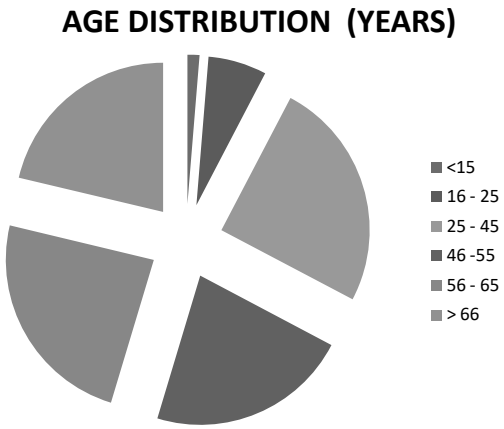


Figure 2

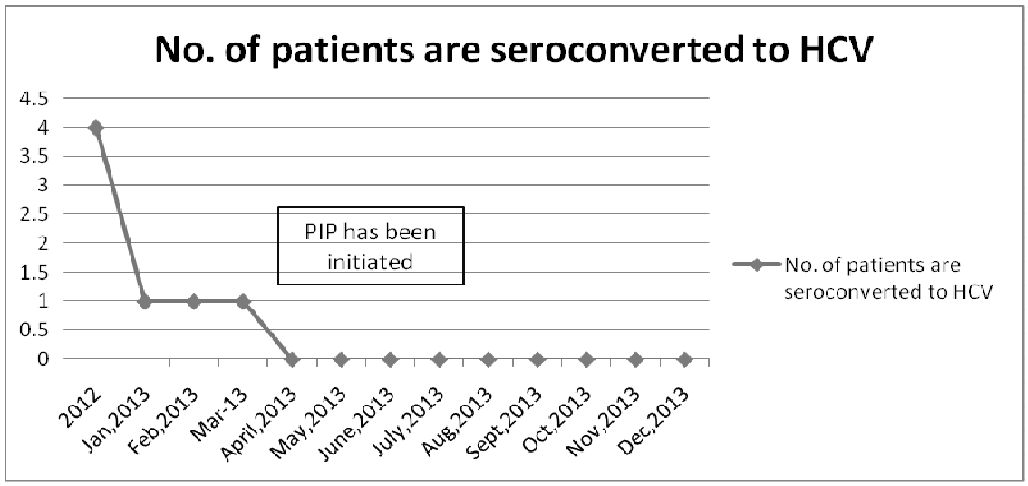


Figure 3

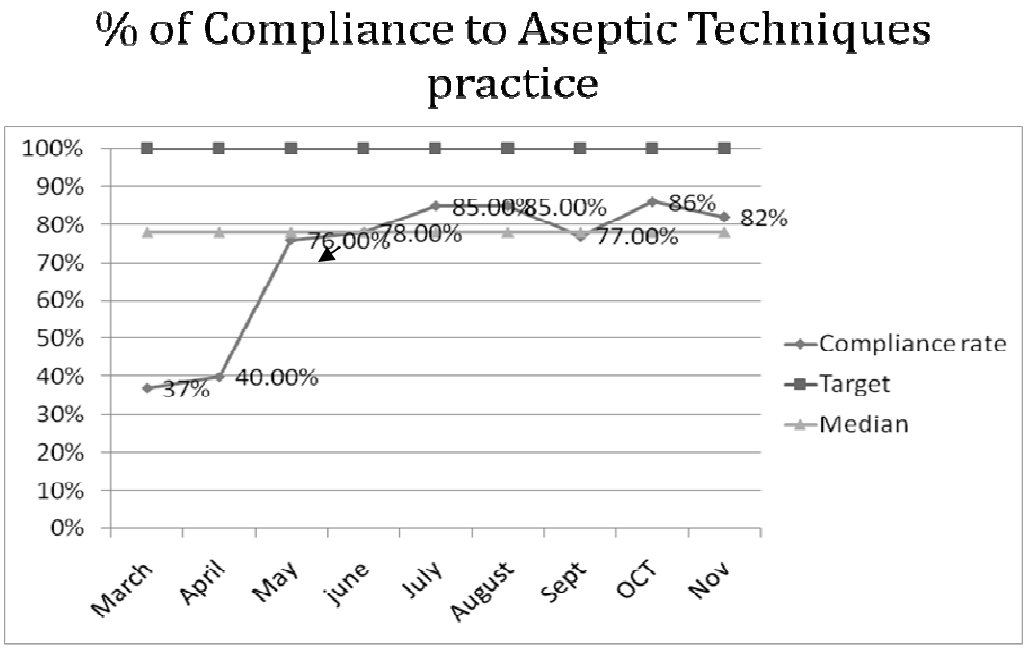
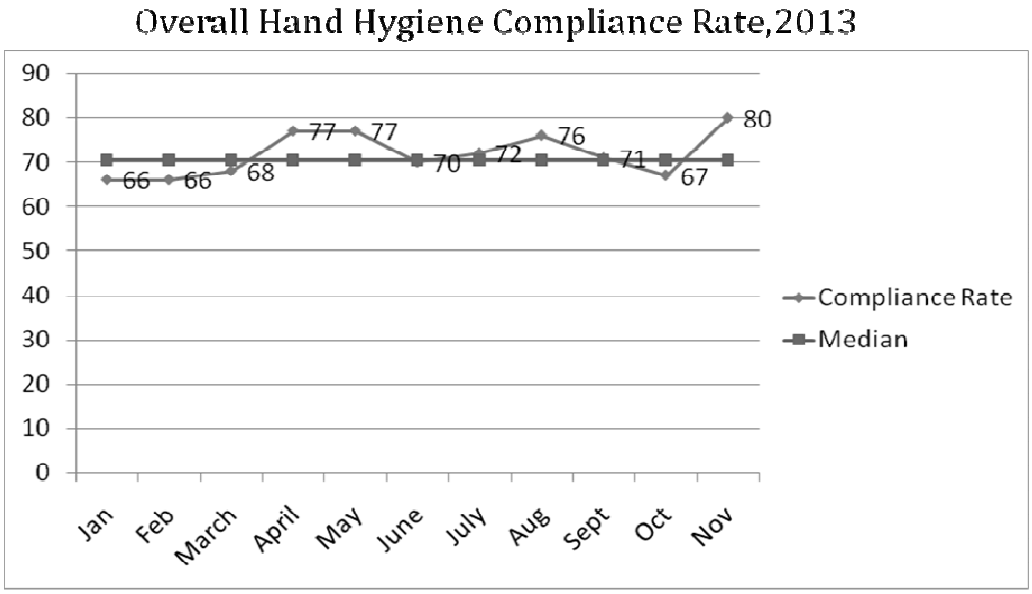


Figure 4:



Discussion:

More than 200 millions of the population (representing 3%) are infected by HCV according the WHO[1]. The prevalence of HCV is much higher in haemodialysis patients than in the general population. Fabrizi confirmed that if the prevalence of HCV in the general population did not exceed 20% in endemic countries, it exceeds in chronic hemodialysis patients 80%[16].

There are many quality control measures were applied to reduce the rates of nosocomial infections in HD patients[12-15].Our present study done in King Fahad Kidney Center (KFKC), from January, 2012 to December, 2013, was applied using these measures aiming to reduce the seroconversion rate of HCV as a nosocomial infection to 0%. The database of this study was assessed, supervised and reported by the quality control center of KFKC.

Outbreaks of hepatitis C has been reported in KFKC. In 2012 four patients (prevalence is 1%) were seroconverted from anti-hepatitis C virus (HCV) negative to anti-HCV positive. On the other hand, in first quarter of 2013, Prevention and Control of Infection Department in KFKC reported three new cases (prevalence in these 3 months only is 0.78%) were seroconverted to HCV positive among hemodialysis patients, however, the last 9 months of the study no cases of HCV seroconversion were recorded.

So with implementation of the quality control measures to our patients in KFKC, the prevalence of HCV seroconversion dropped markedly from 1% at the start of the study in January, 2012 to 0% seroconversion at the end of the study in December, 2013. These results are consistent with other studies implementing the HD quality control measures to minimize HCV seroconversion in HD patients[17-20].

In conclusion of our present study, the implementation of the updated quality control measures to HD patients is highly effective in reduction of the HCV seroconversion rates as a blood borne virus.

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Original Article

Effect of Rapid Eye Movement Sleep Deprivation on Memory in Rats: Role of Brain Derived Neurotropic Factor and Oxidative Stress.

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Abstract:

Background: Several studies have shown that sleep deprivation, especially rapid eye movement (REM) sleep enhances learning and consolidation of some forms of memory. In fact it has been shown that REM sleep is increased after learning sessions. On the other hand, sleep deprivation interferes with learning and memory. The exact mechanisms underlying such disturbances are still not fully elucidated. **Aim:** of the present work was to assess the effect of rapid eye movement sleep deprivation (REMSD) on memory consolidation and to investigate the role of brain derived neurotrophic factor (BDNF) in memory impairment of REM sleep deprived rats and the possible effect of oxidative stress as an underlying mechanism. **Material and Methods:** The study was conducted on 24 adult male albino rats with weights ranging from 200-300 g. Rats were divided into 3 groups (8 rats each). Group I (stress-control group): exposed to same experimental conditions for 6 days without sleep deprivation. Group II (REM deprived group): exposed to 6 days REMSD by multiple platforms method. Group III (recovery control group): exposed to 6 days REMSD then allowed to recover from REM sleep loss for 3 days in their normal home cages. All rats were subjected to daily water maze training using modified Morris water maze to reach a hidden platform in the pool. Immediately after daily training, both groups II and III were deprived from REM sleep using multiple platforms method. At the end of the training period, memory retention was tested. Hippocampal BDNF protein level and oxidative stress markers in brain tissues including reduced glutathione (GSH) and melondialdehyde (MDA) were assayed. **Results:** REM sleep deprived rats showed impairment in memory retention testing in modified water maze task as compared to stress control group. In addition, REMSD resulted in a significant decrease of hippocampal BDNF, brain level of reduced GSH and a significant increase of brain level of MDA as compared to stress control group. Recovery from sleep deprivation resulted in a significant improvement in memory retention testing, hippocampal BDNF and oxidative stress parameters as compared to REM sleep deprived rats. Although recovery from sleep deprivation returned hippocampal BDNF back to basal line, there was a significant decrease in results of memory retention testing, level of GSH and increase of MDA as compared to stress control group. In addition a negative correlation was found between the level of hippocampal BDNF and brain tissue MDA. **Conclusions:** The present study supports the involvement of reduced hippocampal BDNF in the memory deficits induced by REMSD and hypothesis that the increased brain oxidative stress markers may be one of possible mechanisms for reduction of BDNF and subsequently impairment of memory. Sleep recovery after REMSD is effective in reduction of oxidative stress and memory deficit but not to base line emphasizing the importance of implementing prompt treatment of sleep deprivation to prevent memory deficit.

Keywords: REM sleep deprivation, memory impairment, oxidative stress, brain derived neurotrophic factor.

Abbreviations:

REM, rapid eye movement; **REMSD,** REM sleep deprivation; **BDNF,** Brain derived neurotrophic factor; **GSH,** reduced glutathione; **MDA,** malondialdehyde; **LTP,** Long term potentiation; **CREB,** cAMP response element-binding protein; **NMDA,** N- methyl-D-aspartate.

Introduction:

Sleep is defined as a natural and reversible state of reduced responsiveness to external stimuli and relative inactivity, which is important for the maintenance of physiological homeostasis and psychological balance. The disturbance or shortening of normal sleep has recently been shown to be harmful for metabolism and endocrine functions, in a way that resembles the effects of premature aging. Lack of sleep may also result in irritability, fatigue, slurred speech, memory lapses, mood changes, depression and even death due to accidents.[1]

Among the multiple functions of sleep, its role in the establishment of memories seems to be particularly important. Sleep has been shown to promote primarily the consolidation of memory.[2] Previous studies in rats and humans aiming to determine whether different sleep stages have different roles in memory consolidation, mainly focused on rapid eye movement (REM) sleep and the consequences of REM sleep deprivation (REMSD).[3,4] REM sleep was shown to increase after learning a certain task during specific time periods dependent on the task. In the Morris water maze task, it started more than 2 h after learning and persisted for 22 h. The increase in REM sleep during the specific time periods predicted later memory recall, whereas selective deprivation of REM sleep during these time periods was shown to impair memory.[5]

Memory is composed of several stages including acquisition, consolidation (retention) and retrieval. Acquisition is the step during which a task is learnt, consolidation is the process during which the memory is stabilized, and retrieval is the bringing back of the learned task.[2] These three stages of memory can be tested in experimental animals using water maze task.[6]

Memory consolidation involves the strengthening of memory representations through anatomical changes at the synaptic level (synaptic consolidation).[7] Long-term potentiation (LTP) is considered a major mechanism of synaptic consolidation that is induced in the hippocampus during REM sleep. REM sleep has been shown to mediate the expression of plasticity-related immediate early genes (IEGs) like activity regulated cytoskeleton associated protein (Arc), Early growth response protein 1 (Egr1) and brain derived neurotropic factor (BDNF) through hippocampal LTP. Of those, BDNF is especially considered a key protein involved in hippocampal synaptic plasticity. It participates in multiple forms of learning and memory formation[8]. Monfils et al[9] found that consolidation of fear memory increases the binding activity of phosphorylated cAMP response element-binding protein (CREB) to BDNF promoters and upregulates BDNF expression in the amygdala. In addition, hippocampus-dependent contextual learning, or a spatial learning task (water maze task), increases BDNF expression in the hippocampus.[10,11] Upregulation of BDNF has been also observed in the inferior temporal cortex during declarative memory formation[12]. On the other hand, REMSD has been shown to impair the induction of hippocamal LTP and to increase the oxidative stress in the brain resulting in memory impairment.[1,7] However, the exact mechanism underlying memory impairment after REMSD is not fully elucidated.

The aim of the present work was to assess the effect of REMSD on memory consolidation and to investigate the role BDNF in memory impairment of REM sleep deprived rats, and the possible effect of oxidative stress as an underlying mechanism.

Materials and Methods:

The study was conducted on 24 adult male albino rats, with a body weight ranging from 200-300 g. The animals were kept under standard laboratory conditions, maintained on a 12-h light–dark cycle with free access to food and water. Rats were divided into 3 groups. Group I: included 8 rats **(stress-control group),** this group wasn't sleep deprived but rats were exposed to the same experimental conditions by being placed on large platforms in the water pool allowing them to sleep. Group II: included 8 rats that were subjected to REMSD for 6 days **(REM deprivated group)** by multiple platforms method.[13] **Group III:** included 8 rats

(recovery control group) that were subjected to REMSD for 6 days then allowed to recover from REM sleep loss for 3 days in their normal home cages. Behavioral studies were used to assess the effect of REMSD on memory retention follows: Rats were subjected to daily water maze training using modified Morris water maze., immediately after daily training they were deprived from REM sleep using multiple platforms method. At the end of the training period, memory retention was tested.

Water maze task was performed for all rats. The water maze consisted of a dark circular pool, 125 cm in diameter, 55 cm in height, filled with opaque water (about 22° ± 3°C) to a depth of 20 cm., a submerged circular black platform (10 cm in diameter) was placed 20 cm away from the edge in a fixed location and 1 cm below the water surface. The pool was divided into 4 quadrants by 4 starting points marked on its wall: north, south, east, and west (N-S-E-W). The platform provided the only escape from the water. Several cues were placed outside the maze in a fixed position relative to the pool as a window, colored curtain and a red flag; they helped the rat to locate the position of the escape platform hidden below the water surface.[6] The path taken by the animal was recorded by a digital video camera that was mounted above the center of the pool to record the distance traveled and time taken by each rat to reach the platform. The distance was measured by aid of a grid with 10 cm squares placed over the pool (figure 1).[14]

All experimental rats were subjected to daily water maze training before testing of memory retention on last day. During the training session each rat was placed in the water facing the wall of the pool at one of the four designated starting points (north, east, south and west) and allowed to swim and find the hidden platform located in the NW quadrant (target quadrant) of the maze. Each of four starting positions was used once in four training sessions.[15] During each trial, each rat was given 120 seconds (sec) to find the hidden platform. After mounting the platform, the animals were allowed to remain there for 20 sec, and were then placed in a holding cage for 30 sec until the start of the next trial. After completion of training, the animals returned to their home cages. If the rats couldn't find the platform in 120 sec they were placed on it by the examiner and left there for 20 sec. The duration of training varied according to the studied group. It continued for 6 days for groups I and II and 9 days for group III (6 days REMSD + 3 days recovery).

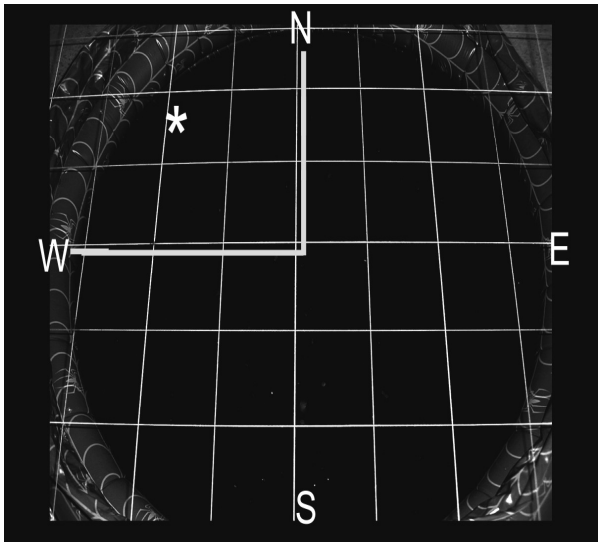

Fig 1: Water maze pool with 4 starting points (N-S-E-W) dividing it into 4 quadrants. The mark points to the location of the hidden platform in the target quadrant of the pool (N-W). A grid with 10 cm squares was placed over the pool to measure the distance swum by the rat.

I- Memory retention testing:

At the end of the training period memory retention testing was performed 24 hours after last day of training for each group, to test for memory consolidation. Retention testing consisted of 60 sec of a free swimming period for each rat with the hidden platform removed from the pool. The better the memory is consolidated the more time will the rat swim around the location of the platform and less escape trials will be attempted from the wall of the pool. The time spent in the target quadrant [expressed as percent of the time spent in the pool (total of 60 sec)], the distance swum in the target quadrant (expressed as percent of the distance swum in the pool) and the number of escape trials from the edges of the pool were calculated.(15)

II- REM sleep deprivation procedure:

REMSD began immediately after each daily training session using the modified multiple platforms method.(1)

Stress control group was submitted to the same procedure, except the platforms were 13 cm in diameter allowing them to sleep on it. Except for the size of the platform, all other conditions remained identical to that of the experimental animals.(16)

Recovery control group included those animals that had been REM sleep deprived and then allowed to live in normal cages for 3 days to recover from lost REM sleep.(16)

The modified multiple platforms method involved placing the rats inside a circular water tank (125×55 cm), containing 12 circular platforms, 6.5 cm in diameter, with water up to 1 cm of their upper surface. The rats could thus move around inside the tank by jumping from one platform to another. When they reached the REM phase of sleep, muscle atonia set in and they fell into the water and woke. Food and water were provided for all groups through placing food on the platforms inserted in the centre of the pool close to the other platforms. The water in the tank was changed daily, throughout REMSD period.(1,3)

III- Biochemical studies:

On the last experimental day for each group; the rats were sacrificed by decapitation immediately after behavioral assessments. The whole brain was removed and washed with ice cold saline. Hippocampus was dissected through the following way: a small curved forceps was placed between the cerebral halves in a closed position, and then the forceps was repeatedly opened and closed. Once an opening was obtained along the midline, the forceps was opened and directed to both sides to separate the cortex from the hippocampus. This movement was repeated on either side until the upper part of the hippocampus was visible (identified by its whitish color). The cortex was gently picked up with the forceps and the hippocampus was freed from the cortex without damaging the cortex. The hippocampus was cut and separated from the fornix. The two halves of the hippocampus were separated and the hippocampus on one side was rolled out of the brain to remove it from the cortex.(17) Hippocampus and the remaining brain tissue from each rat were stored at a temperature of -80 ^{o}C for biochemical analysis.

At the time of analysis the hippocampus and remaining brain tissues for each rat were removed from the freezer. Lysis buffer (137mM NaCl, 20mM Tris–HCl pH 8.0, 1% NP-40, 10% glycerol, 1mM phenylmethylsulphonyl fluoride, 10 μgml^{-1} aprotinin, 1 μgml^{-1} leupeptin, 0.5 mM sodium vanadate) was added to each sample (2 samples for each rat one for hippocampus and the other for remaining brain tissues). After that all the samples were homogenized. The homogenate was centrifuged at 12,000 rpm for 20 min at 4°C to remove tissue debris. The supernatant was aliquoted and frozen at -80°C until analysis.(18)

The protein concentration of each sample was measured in supernatants of hippocampus and the remaining brain tissues using lowry method(19) and was expressed in mg/ml. Supernatant from hippocampus homogenate was used for estimation of BDNF protein level by the enzyme-linked immunosorbent assay (ELISA) kit (Boster Biological Technology., LTD).(18) Its concentration in the hippocampus was divided by the concentration of protein (mg/ml) to be finally expressed as pg/mg protein. The minimum detectable dose of rat BDNF was less than 2 pg/ml. No detectable cross- reactivity with any other cytokine was detected by the manufacture. Supernatants from brain tissues homogenate were used for estimation of:

Reduced glutathione level (GSH) which was estimated by colorimetric method and was finally expressed as ng GSH/mg protein of the remaining brain tissue;[20] as well as malondialdehyde (MDA) level which was estimated by colorimetric method. Lipid peroxidation was finally expressed as nmol MDA/mg protein of the remaining brain tissue.[21]

Statistical analysis:

The values of the measured parameters were expressed as mean±SD. The difference between the studied groups was determined using ANOVA test (F-test) and least significant difference (LSD). Pearson's correlation coefficient was performed for evaluating the behavioral, neurochemical and biochemical variables. P<0.05 values were considered significant. All statistical analyses were processed using SPSS for windows Version 18.

Results:

Comparison between different studied groups regarding memory retention testing:

Water maze memory retention testing after 6 days REMSD in group II caused a significant decrease in the percent of the time spent in the target quadrant of the pool as compared to stress control group where p<0.0001. Recovery from REMSD in group III caused a significant increase in the percent of the time spent in the target quadrant of the pool as compared to group II where p<0.0001. However, the percent of time spent in the target quadrant in group III was still significantly less than in the stress control group where p<0.001.

In addition, 6 days REMSD in group II caused a significant decrease in the percent of the distance swum in the target quadrant of the pool as compared to stress control group where p<0.0001. Recovery from REMSD in group III caused a significant increase in the percent of the distance swum in the target quadrant of the pool as compared to group II where p<0.05. However, the percent of distance swum in the target quadrant in group III was still significantly less than that stress control group where p<0.001 indicating incomplete recovery of memory.

Moreover, REMSD for 6 days in group II caused a significant increase in the number of escape trials from the edges of the pool as compared to stress control group where P<0.05. Recovery from REMSD in group III caused no change in the number of escape trials as compared to group II. However, the number of escape trials in recovery control group was significantly higher than the stress control group where p<0.01. (Table 1)

Hippocampus BDNF (pg/mg protein):

The mean values for hippocampus BDNF in group I, II and III were 5335±942.7 (pg /mg protein), 3377±1781.4 (pg /mg protein) and 5570±1783.6 (pg /mg protein) respectively. There was a significant difference between the groups where F value was 16.98 and p < 0.001.

In group II, 6 days REMSD caused a significant decrease in hippocampus BDNF as compared to stress control (group I) where p<0.05. Recovery from REMSD in group III caused a significant increase in hippocampus BDNF as compared to group II where p<0.05. However, recovery from REMSD caused no change in hippocampus BDNF as compared to stress control group. (Table 2)

Brain tissue GSH (ng/mg protein):

The mean values for brain tissue GSH in group I, II and III were 9.88±1.35 (ng/mg protein), 6.96±1.18 (ng/mg protein) and 8.38±1.48 (ng/mg protein) respectively. There was a significant difference between the groups where F value was 16.85 and p<0.001.

REMSD for 6 days in group II resulted in a significant decrease in GSH as compared to stress control (group I) where p<0.0001. Recovery from REMSD in group III caused a significant increase in GSH as compared to group II where p<0.05. In addition, recovery from REMSD caused a significant decrease in GSH as compared to stress control group where p<0.05 indicating incomplete return of brain tissue GSH to basal line. (Table 3)

Brain tissue MDA (nmol /mg protein):

Brain tissue MDA mean values in Stress-control, REM deprivated and the recovery control groups were 0.49±0.29 (nmol /mg protein), 3.75±1.1 (nmol/mg protein) and 1.29±0.9 (nmol/mg protein) respectively. There was a significant difference between the groups where F value was 16.98 and p<0.001.

It was observed that 6 days REMSD in group II caused a significant increase in brain

tissue MDA as compared to stress control (group I) where p<0.0001. Recovery from REMSD in group III caused a significant decrease in brain tissue MDA as compared to group II where p<0.0001. In addition, Recovery from REMSD caused a significant increase in brain tissue MDA as compared to stress control group where p<0.05 indicating incomplete return of brain tissue MDA to basal line. (Table 4)

Correlation study:

I- Correlation study between level of hippocampus BDNF and performance in memory retention testing:

A significant positive correlation was found between level of hippocampus BDNF and the percent of time spent in the target quadrant of the pool where correlation coefficient r = 0.468 and P<0.05 (figure 2).

In addition, a significant negative correlation was found between level of hippocampus BDNF and number of escape trials from edges of the pool where correlation coefficient r = -0.401 and p<0.05 (figure 3).

II. Correlation between level of hippocampus BDNF and brain tissue MDA

A negative correlation was found between level of hippocampus BDNF and brain tissue MDH where correlation coefficient r = -0. 523 and p < 0.05 (Figure: 4).

Table (1): Comparison between different studied groups of albino rats regarding memory retention testing

	Group I (Stress control)	Group II (REM deprived)	Group III (Recovery control)	F test (P value)
Percent of time spent in target quadrant	38.13±6.45	7.50 ± 3.98• •p<0.0001	17.71 ± 9.47•# •p < 0.001 #P < 0.0001	F= 26.85 (p<0.0001*)
Percent of distance swum in target quadrant	60.34±18.57	15.32±4.34• •p<0.0001	28.98±15.56•# •p<0.001 #P<0.05	F=25.65 (P< 0.0001*)
Escape trials	1.12 ±1.12	6.50±5.18• •p<0.05	4.50 ± 2.82• •p<0.005	F=14.25 (P <0.01*)

Data are presented as mean±SD *significant • Significant vs group I, #significantvs group II

Table (2): Hippocampus Brain derived neurotrophic factor (BDNF) (pg /mg protein) in the different studied groups of albino rats

BDNF (hippocampus) (pg /mg protein)	Group I (Stress control)	Group II (REM deprived)	Group III (Recovery control)	F test (P value)
Min- Max **Mean ± SD**	3731- 6358 5335 ± 942.7	1261- 6420 3377±1781.4• •p< 0.05	2092- 7983 5570±1783.6# #p< 0.05	16.98 (P < 0.001*)

Data are presented as mean±SD *significant • Significant vs group I, # significant vs group II

Table (3): Brain tissue reduced glutathione (GSH) (ng/mg protein) in the different studied groups of albino rats.

GSH (ng/mg protein)	Group I (Stress control)	Group II (REM deprived)	Group III (Recovery control)	F test (P value)
Min- Max **Mean± SD**	8.20 -11.62 9.88 ±1.35	6.10 - 9.28 6.96±1.18• •p<0.0001	6.37-10.83 8.38±1.48•# •p<0.05 #p<0.05	16.85 (P<0.001*)

Data are presented as mean ± SD *significant p • Significant vs group I, #significant vs group II.

Table (4): Brain tissue melondialdehyde (MDA) (nmol /mg protein) in the different studied groups of albino rats

MDA (nmol/ mg protein)	Group I (Stress control)	Group II (REM deprived)	Group III (Recovery control)	F test (P value)
Min- Max	0.21- 0.90	2.42 - 5.89	0.21- 2.50	16.98
Mean± SD	0.49 ± 0.29	3.75 ± 1.1•	1.29 ± 0.9•#	(P<0.001*)
		•p<0.0001	•p < 0.05	
			#p<0.0001	

Data are presented as mean ± SD * Significant • Significant vs group I, # significant vs group II.

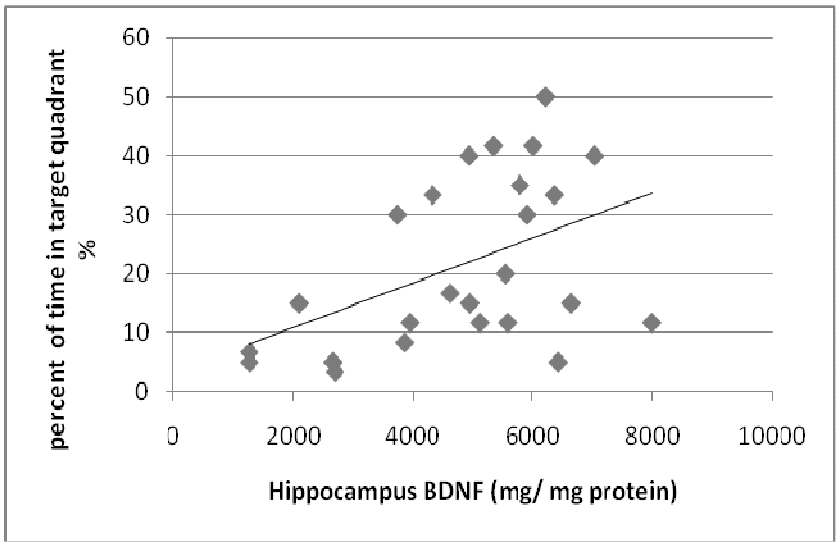

Figure 2: Correlation between level of hippocampus BDNF and performance in memory retention testing as regard the percent of time in the target quadrant of pool in albino rats

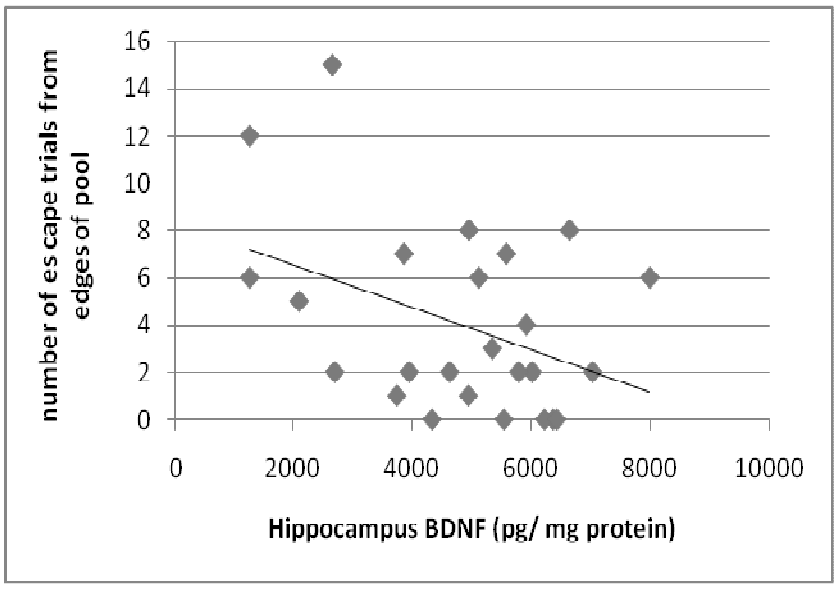

Figure 3: Correlation between level of hippocampus BDNF and performance in memory retention testing as regard the number of escape trials in albino rats.

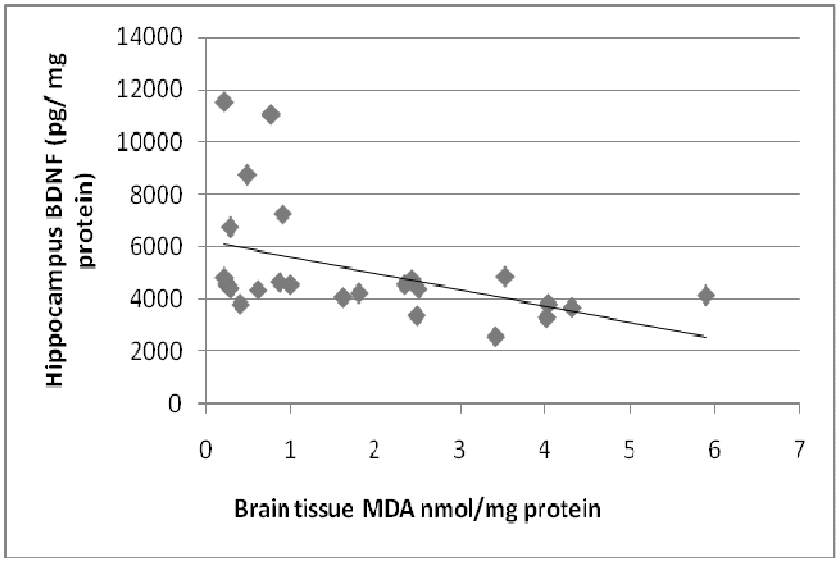

Figure 4: Correlation between level of hippocampus BDNF and brain tissue MDA in albino rats

Discussion:

Sleep loss is a common feature of many sleep disorders in humans, and for this reason analyses of behavioral and neurochemical effects seen in animal models of sleep deprivation are of considerable interest. Several studies (2,22) have shown that sleep (especially REM sleep) enhances learning and consolidation of some forms of memory. In fact it has been shown that REM sleep is increased after learning sessions.[23] On the other hand, sleep deprivation interferes with learning and memory,[24,25,26] however the exact mechanisms underlying such disturbances are still not fully elucidated.

In the present work results of water maze memory retention testing revealed that REMSD caused a significant decrease in the percent of the time spent and percent of the distance swum in the target quadrant of the pool as compared to the stress control group. It also caused a significant increase in the number of escape trials from the edges of the pool as compared to the stress control group signifying an impairment of memory retention and retrieval. In agreement with these results are the findings of Yang et al[27] who found that rats subjected to 4 hours of daily sleep deprivation for 7 days traveled a longer distance to find the hidden platform during the acquisition training and had fewer numbers of platform crossings in the memory retention testing than those in the control group during water maze training. Similarly, Kalonia et al[1] found that rats deprived of REM sleep for 72 h using a grid suspended over water, showed impairment in memory retention testing by using elevated plus maze, passive avoidance and Morris water maze tests. In addition, Alvarenga et al[3] studied the effects of REMSD for 96 h on the learning/memory processes in rats submitted to the plus-maze discriminative avoidance task, which simultaneously evaluates learning, memory, anxiety and motor function and concluded that REMSD impaired acquisition, consolidation, and recall of a discriminative avoidance task in rats.

In the present work, when rats were allowed to recover from sleep deprivation, memory retention testing revealed an improvement in memory consolidation in recovery control group as compared to REM sleep deprived group, however memory performance was less than stress control group, indicating that the improvement in memory after recovery from REMSD didn't return to baseline. Similarly, Li et al[28] found that REMSD for 48 h produced a significant impairment in retention of acquired spatial reference memory of young rats in a Morris water maze, and impairment continuously existed after 24h and 48h of recovery from sleep deprivation which indicates that REM sleep play a critical role in memory maintenance and consolidation and REMSD may play a role in long-term impairment in the

consolidation of newly acquired memories which are hippocampus dependant. The role of REM sleep in hippocampus dependant memory consolidation has been further investigated by smith et al[23] who found that REMSD disrupts memory consolidation in hidden platform version which is hippocampus dependant, while the visible platform version was not affected by REMSD as it is hippocampus independent. Furthermore other studies indicate that the firing patterns of neurons in the hippocampus during prior spatial learning tasks were found to replay during subsequent REM sleep.[29,30] In addition, Yang et al[31] demonstrated that REMSD disturbed the rhythmic activity of theta band in the hippocampus, which may be involved in the deficit of the spatial learning and memory retention testing in rats. The above studies suggest that memory consolidation in the hippocampus is affected by REMSD. Such findings prompted us to investigate the effect of REMSD on hippocamal BDNF and its relation to learning and memory deficit. BDNF is a neurotrophin that enhances the growth and differentiation of neurons and synapses.[32] In the CNS, BDNF is functionally active in the hippocampus, cortex, and forebrain, the areas that have a vital role in learning and memory.[33] The present work demonstrated that 6 days REMSD caused a significant decrease in hippocampus BDNF as compared to stress control. Recovery from REMSD caused a significant increase in hippocampus BDNF as compared to REM sleep deprived group; however, the change was insignificant when compared to the stress control group. Our findings are in agreement with Guzman-Marin et al[18] who compared the effects of short- term (8 h) and intermediate-term (48 h) sleep deprivation on the expression of BDNF in the neocortex and the hippocampus. They showed a significant decrease of mRNA of BDNF and its protein level in the hippocampus. However, neither 8 h nor 48 h sleep deprivation or control treatments had significant effects on BDNF in the neocortex. The reduction in the levels of BDNF in the hippocampus after REMSD is consistent with a finding showing that sleep deprivation reduces the protein levels of the phosphorylated form of the extracellular signal-regulated kinase 2 (phospho-ERK2), which is one of the sequences of the BDNF receptor activation.[24] Furthermore, in the present study there was a significant positive correlation between BDNF and percent of time spent in the target quadrant; in addition, there was a negative correlation between BDNF and the number of escape trials from the edges of the pool in all studied groups. These findings demonstrate the relation between BDNF and memory retention testing, where rats spent less time in the target quadrant and had more tendencies to escape from the pool as the level of BDNF decreased.

The effect of sleep deprivation induced decrease in BDNF on memory retention and consolidation could involve hippocampal synaptic plasticity. One prominent form of hippocampal synaptic plasticity is LTP, a long-lasting change in the strength of synaptic connections that is a frequently used model to study the mechan isms underlying learning and memory.[34,35] In rats, the use of forced locomotion to induce total sleep deprivation for 12 h or sleep fragmentation for 24 h, was found to impair hippocampal LTP recorded from Schaffer collateral CA1 synapses. Similar LTP deficits were also produced by REMSD using the platform-over-water technique for 24 to 75h in area CA1 both in vitro and in vivo[36,37] In addition, Marks and Wayner[38] showed that a single sleep disruption of REM sleep for 3-h reduced dentate granule cells LTP in anesthetized rats. These studies indicate that REMSD impaired LTP in CA1 and dentate granule cells of hippocampus. N- methyl –D-aspartate (NMDA) receptors are mediators of LTP induction and spatial memory in pyramidal neurons in CA1 region of the hippocampus. Numerous studies blocking NMDA receptor activity or knocking out genes for selected subunits have been carried out and have lead to reduced LTP induction and impaired spatial memory[39,40]. On the other hand, overexpression of the NR2B subunit of the NMDA receptor leads to an enhancement of learning and memory in mice[41]. McDermott et al[34] found that 72h of REMSD results in a lower density of surface NMDA receptors; whereas modifications that enhance NMDA receptor activity restore the ability of these synapses to undergo potentiation. These observations may explain the reduced ability to induce LTP due to reduction of BDNF which is considered a key protein in hippocampal synaptic plasticity. This may be considered as one of mechanisms that explain sleep deprivation-induced memory impairments.

Several explanations have been proposed for the decrease of BDNF during REMSD, it

was suggested that the decrease in BDNF could be attributed to decrease of hippocmpal theta wave activity that was shown to occur in REMSD. Theta wave activity in REM sleep is thought to enhance hippocampus LTP, BDNF gene expression and its release from stored vesicles.[42] It has also been suggested that sleep deprivation-induced memory deficits might possibly be due to the combined effect of oxidative damage and alterations in several neurotransmitter levels.[1] Therefore in the present study we measured oxidative stress markers to investigate their role as a possible mechanism for reduction of BDNF and subsequently impairment of memory. We found that REMSD for 6 days caused a significant decrease of GSH and an increase of MDA as compared to the stress control group. In addition, Recovery from REMSD caused a significant increase in GSH and decreased MDA as compared to REM sleep deprived group. However, the recovery caused a significant decrease in GSH and an increase in MDA as compared to stress control group, indicating that recovery from sleep deprivation didn't return measured oxidative measures to basal line. Similarly, Khadrawy et al[43] suggested that the hippocampus is more vulnerable to oxidative stress resulting from REMSD. Their finding was supported by the significant increase in nitric oxide in the hippocampus after 72 h of sleep deprivation. Kalonia et al[1] found that 72 h REM deprivation caused a significant decrease in the levels of endogenous antioxidants such as reduced glutathione and catalase, and an increase in the levels of lipid peroxidation and nitrite, resulting in the generation of free radicals, leading to oxidative stress in the brain.

In the current work, a negative correlation was found between level of hippocampus BDNF and brain tissue MDA. Increase oxidative stress may lead to reduction of BDNF. Similarly, Kapczinski et al,[44] found that alterations in oxidative status may be mechanistically associated with abnormal low levels of BDNF observed in individuals with bipolar disorder. The above findings agree with previous studies that showed that increase oxidative stress may lead to reduction of BDNF.[44,45] Several mechanisms by which oxidative stress could decrease BDNF have been suggested, including decreased CREB[46] which is associated with reduction of BDNF gene expression. In addition, increase oxidative stress results in energy depletion and then impairs NMDA channel function. The impairment of NMDA channel function leads to reduction of BDNF gene expression.[44]

Sleep normally involves a process of detoxification at the cellular level. Reimund[47], concluded that free radicals accumulate during wakefulness and are removed during sleep. Their removal during sleep is accomplished by the efficiency of endogenous antioxidant mechanism. Thus sleep essentially has antioxidative role. In addition, the brain has been shown to be more sensitive to oxidative damage as a result of the abundance of polyunsaturated fatty acids, deficient antioxidant defense systems, and a high rate of energy use resulting from the higher metabolic rate.[48] Sleep deprivation on the other hand has been shown to cause oxidative stress resulting in the formation of reactive oxygen species and eventually leading to neuronal and cellular damage, and reduces endogenous antioxidant defense in areas of the brain responsible for memory, particularly the hippocampus, which may be partially responsible for learning and memory deficits.[49,50] Increased brain oxidative stress seems to have an important role in cognitive impairment caused by normal aging and neurodegenerative diseases such as Alzheimer's disease, treatment of chronic sleep deprivation may be of value in preventing such disease.

Conclusions: The present study supports the involvement of reduced hippocampal BDNF in the memory deficits induced by REMSD, and hypothesis that the increased brain oxidative stress markers may be one of possible mechanisms for reduction of BDNF and subsequently impairment of memory. Sleep recovery after REMSD is effective in reduction of oxidative stress and memory deficit but not to base line emphasizing the importance of implementing prompt treatment of sleep deprivation to prevent memory deficit.

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