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Assessment of Advanced Glycation Endproducts in Offspring of Type 2 Diabetes

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Abstract: ***Background: Diabetes is a group of metabolic diseases characterized by hyperglycemia, There are several, well researched theories of how chronic hyperglycemia can lead to micro or macrovascular disease in diabetes including the advanced glycation end products (AGEs) theory.pentosidine is one of the best chemically characterized AGEs in humanspentosidine levels were significantly increased in diabetic patients with retinopathy and in diabetic patients with nephropathy. Aim of the work: This study is designed to identify any changes of the level of AGEs in offspring of type 2 diabetes with and without impaired glucose tolerance and its relation to age, body mass index and various metabolic parameters in those subjects. Subjects: Case control study was carried out On 66 volunteers subjects. And were divided into two main groupswhich were age, sex matched:Group A Control group, includes 33 apparently healthy subjects. Group B: Includes 33 non diabetic offspring of type 2 diabetic patientsof whom one or both parents are diabetic which were further subdivided into 2 subgroups according to oral glucose tolerance test into: i) Subgroup B1:Non diabetic normal glucose tolerant offspring, includes 16 healthy subjects. ii)Subgroup B2:Non diabetic impaired glucose tolerant offspring, includes 17 healthy offspring subjects. Methods: all subjects of study were subjected to full history and thorough clinical examination, routine investigation as urine analysis, complete blood picture, liver and renal function tests oral glucose tolerance test, lipid profile .And specific investigation included measurement of fasting insulin and serum pentosidineby ELISA technique, and calculation of insulin resistance by HOMA index. Results: There is significant increase in serum pentosidinelevel in group B2 in comparison to group A and group B1while there is no significant difference in this risk factor between Group A and Group B1. There is significant positive correlation between serum pentosidine level and each of fasting insulin level and HOMA-IR in group B2. While there was no significant correlation between pentosidine and each of age, Smoking status, BMI, waist circumference, cholesterol, triglycerides, FBG and 2hr postprandial blood glucose in group B1and groupB2. Conclusion:the high level of serum pentosidine as an advanced glycation end product (AGE) in offspring of type 2 diabetes with impaired glucose tolerancemay play a role in many vascular complications that occur before development of diabetes. Therefore we recommend screening offspring of type 2 diabetes for AGEs and early management to guard against vascular complications that may occur in the pre diabetic state.***

Keywords:

1. Introduction

Diabetes mellitus, especially type 2 diabetes (T2DM), is increasing at an alarming rate and is considered as one of the main threats to human health in the 21st century, in both developed and developing nations **(34)**.

The pathogenesis of T2DM is complex and involves the interaction of genetic and environmental factors. Individuals with T2DM show both insulin resistance and beta cell defects. Insulin resistance means that there is decreased ability of target organs like liver, adipose tissues and skeletal muscles to respond to normal circulating concentration of insulin. Post binding defects in insulin action are primarily responsible for insulin resistance in T2DM**(3)**.

Prolonged exposure to hyperglycemia is now recognized as the primary causal factor in the majority of diabetic complications. There are several, well researched theories of how chronic hyperglycemia can lead to micro or macrovascular disease in diabetes including the advanced glycation end productss (AGEs) theory [28].

AGEs constitutes a large group of heterogeneous molecules formed by the non enzymatic reactions of sugar with free amino groups of proteins, lipids, peptides and nucleic acids within the so called "Maillard reaction", a tribute to the French scientist Louis Camille Maillard (1878–1936) [21].

Tissue AGEs accumulation is thought to be specific marker of long term glycemic control, oxidative stress and cardiovascular risk. Prediabetes (impaired fasting glucose (IFG) and impaired glucose tolerance (IGT)) are considered as risk categories for the development of both type 2 and cardiovascular disease**(10)**.

Many in vitro and in vivo studies as well studies using anti AGE agents have demonstrated that these chemically heterogeneous compounds are known to have a wide range of chemical, cellular and tissue effects implicated in the development and progression of diabetic complications**(25)**.

Pentosidine is one of the best chemically characterized AGEs in humans and acts as a marker for the formation and accumulation of AGEs[13].Pentosidine was characterised as a sensitive marker for all AGEs**(8)**.

Interaction of pentosidine with the receptor for AGEs (RAGEs) causes activation of intracellular signaling, gene expression, and production of pro inflammatory cytokines and free radicals[1].

Pentosidine levels were significantly increased in diabetic patients with retinopathy and in diabetic patients with nephropathy.Pentosidine levels were increased markedly with the severity of microangiopathy[19].

Aim:
This study was performed to studyany changes of the level of AGEs in offspring of type 2 diabetes with and without impaired glucose tolerance and its relation to age, body mass index and various metabolic parameters in those subjects.

2. Patients and Methods

a) Subjects
Case control study conducted in the Internal Medicine and Medical Biochemistry departments, Faculty of Medicine, Zagazig University, from April 2015 to October 2015. Our studyincluded a total number of 66 volunteers subjects. They were chosen after their own written consent and were divided into two main groups which were age and sex matched as follow:

1) Group A:
Control group, includes 33 apparently healthy subjects (20 males and 13 females).Their ages ranged from 19 years to 38 years with mean values ±SD (27.82 ± 4.92) years, their BMI ranged from 19 to 28 kg/m^2 with mean values ±SD of (23.48 ± 2.36), and non diabetic (their fasting blood glucose level ranged from (85mg/dL) to (99mg/dL) with mean values±SD of (93.6 ± 4.4mg/dL) and their 2hours postprandial blood glucose level ranged from (115 mg/dL) to (139 mg/dL) with mean values ±SD of (129.6 ± 6.8mg/dL), 12 of them are smokers. They had no family history of diabetes mellitus, hypertension or obesity.

2) Group B:
Includes 33 non diabetic offspring of type 2 diabetic patients of whom one or both parents are diabetic on insulin or oral hypoglycemic drugs which were further subdivided into 2 subgroups according to oral glucose tolerance test into:

i) Subgroup B1:
Non diabetic normal glucose tolerant offspring, includes 16 subjects (9 males and 7 females).Their ages ranged from 19 years to 33 years with mean values ±SD (26.13 ± 5.05) years, their BMI ranged from 20 to 28 Kg /m^2 with mean values ±SD of (23.75 ± 2.97), and with normal glucose tolerance (their fasting blood glucose level ranged from (85 mg/dL) to (99mg/dL) with mean values ±SD of (93.6 ± 4.6 mg/dL) and their 2hours postprandial blood glucose level ranged from (120 mg/dL) to (139 mg/dL) with mean values ±SD of (130.7 ± 5.4 mg/dL), 5 of them are smokers.

ii) Subgroup B2:
Non diabetic impaired glucose tolerant offspring, includes 17 healthy subjects (7 males and 10 females).Their ages ranged from 19 years to 40 years with mean values ±SD (30.24 ± 6.97) years, their BMI ranged from 20 to 31 kg/m^2 with mean values ±SD of (26.41 ± 3.50), and with impaired glucose tolerance (their fasting blood glucose level ranged from (108 mg/dL) to (125 mg/dL) with mean values ±SD of (118.7 ± 5.4 mg/dL) and their 2hour postprandial blood glucose level ranged from (163 mg/dL) to (197 mg/dL) with mean values ±SD of (181 ± 10.6 mg/dL), 3 of them are smokers.

b) Inclusion Criteria
1) Age: Subjects > 18 years of either sex.

Classification	*BMI (Kg/m^2)*
*Under weight	< 5.81
*Normal range	18.5 – 24.9
* over weight	25-29.9
* Obese class I	30 – 34.9
* Obese class II	35 – 39.9
* Obese class III	≥ 04

2) Offspring of type 2 diabetic patients of whom one or both parents are diabetic on insulin or oral hypoglycemic drugs with normal or impaired glucose tolerance (normal glucose tolerance diagnosed by fasting blood glucose < 100 mg/dl, and 2 hours post-prandial<140mg/dl & impaired glucose tolerance diagnosed by fasting blood glucose 100-125mg/dL, and/or 2 hours post prandial 140-199mg/dl).
3) Subjects with normal glucose tolerance & negative family history of diabetes as control group.
4) Adequate hepatic, renal, cardiac and respiratory functions.

c) Exclusion criteria:
1) Uncontrolled Hypertension.
2) Presence of Liver and/or Kidney diseases.
3) Thyroid, Cardiovascular or any active inflammatory diseases.
4) Overt diabetes with fasting blood glucose ≥ 126 mg/dl and/or 2-h post prandial glucose level ≥ 200 mg/dl.
5) Pregnant ladies and those receiving contraceptive pills.

d) Methods:
All subjects of the study were subjected to the following:

1) Full history and thorough clinical examination:
With special stress on:
a) Family history of diabetes mellitus, hypertension and obesity.
b) Calculation of the body mass index (B.M.I):
BMI, also called Quetelet's index was derived by dividing weight by the square of height.

$$BMI = \frac{\text{Weight (Kg)}}{\text{Height (m)}^2} = \text{Kg / m}^2$$

Over weight is defined as BMI > 25 and obesity as BMI > 30[17]. This classification was subsequently adopted by the National Institutes of Health [26], and now has become a worldwide standard.
Classification of obesity based on BMI (Table:1) (WHO, 1997)
c) Calculation of the waist circumference
The waist circumference is measured at a level midway between the lowest rib and the iliac crest[14].
Abdominal obesity is defined as a waist circumference ≥ 102 cm in men and ≥ 88 cm in women[27].

2) Routine investigations:
(to verify the inclusion and exclusion criterias of studied subjects) and include:

a) **Urine analysis:** (for glucose, acetone, protein, pH, bilirubin and leukocytes) by urinestrips.
b) **Complete blood picture:** By automated blood counter.
c) **Liver function tests:** (serum bilirubin (total and direct), serum albumin, serum ALT and AST) by colorimetric method[30].
d) **Renal function tests:** serum creatinine[15]and serum urea by colorimetric method[12].
e) **Electro Cardio Gram(ECG):**
f) **Oral glucose tolerance test:**

- A 75-gram oral glucose tolerance test was performed after an overnight fast.Venous plasma glucose samples were analysed by the hexokinase method[4].
- Glucose tolerance was classifiedinto:
  - **a)** Normal glucose tolerance (**NGT**):
    Fasting glucose:< 100 mg/dl (5.6 mmol/l)
    Post load glucose:< 140 mg/dl (7.8 mmol/l)
  - **b)** Impaired fasting glycemia(**IFG**):
    Fasting glucose: ≥ 100 mg/dl (5.6 mmol/l) but <126 mg/dl (7 mmol/l)
    Post load glucose:<140 mg/dl (7.8mmol/l)
  - **c)**Impaired glucose tolerance (**IGT**):
    Fasting glucose:<100 mg/dl (5.6mmol/l)
    Post load glucose: 140 mg/dl (7.8mmol/l) -199 mg/dl (11mmol/l)
  - **d)** Diabetes:
    Fasting glucose: ≥ 126 mg/dl (7mmol/l)
    Post load glucose: ≥ 199 mg/dl (11mmol/l)[2].

3) **CRP.**

I) Specific investigations included:

1. Measurment of fasting insulin by ELISA technique (µIU/ml)

Was done by Insulin AccuBind ELISA Kit produced by Monobind Inc. Lake Forest, CA 92630, USA, Product code: 5825-300. Monobind Insulin Microplate Elisa test is intended to be used for the quantitative determination of insulin levels in human serum.

Principle of Test

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (Ab), (enzyme conjugated and immobilized), with different and distinct epitope recognition, in excess, and native antigen (Ag). In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal Insulin antibody. Upon mixing monoclonal biotinylated antibody, the enzyme labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex[32]**).**

2. Calculation of insulin resistance

By homeostasis model assessment (HOMA IR)[22] index was calculated by the following formula:

HOMA index =

$$\frac{\text{fasting insulin (µIU/ml)} \times \text{fasting plasma glucose (mmol/ l)}}{22.5}$$

Subjects were categorizes as insulin resistant if their HOMA IR was greater than 1.64[6]**.**

4) Measurement of serum pentosidine(ng/ml)

Was done by pentosidine ELISA kit produced by Wuhan El Aab science co. Wuhan. CHINA. Serum values of pentosidine was measured using high sensitivity enzyme linked immunosorbent assay (ELISA)**.**

Principle of test:

ELISA is based on the competitive binding enzyme immunoassay technique. The micro titer plate provided in the kit has been pre coated with an antibody specific to pentosidine. During the reaction pentosidine in the sample competes with a fixed amount of biotin-labeled pentosidine for sites on pre coated Monoclonal antibody specific to pentosidine. Excess conjugate and unbound sample are washed from the plate. Next, Avidin conjugated to Horseradish eroxidase (HRP) is added to each micro plate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is trminated by addition of sulphuric acid solution and the color change is measured spectrophotometrically at awavelengh of 450nm±2nm.The concentration of pentosidine in the samples is then determined by comparing the O.D.of the samples to the standard curve.

Sampling:

After an overnight fasting (8hs), venous blood (8ml) was collected from all subjects under complete aseptic conditions and divided into 3portions:

1) 1ml blood collected on sodium fluoride-oxalate was centrifuged and plasma was separated for fasting plasma glucose and 3 ml were collected for routine lab.
2) 4 ml blood collected on plain tube was left for 30 minutes and centrifuged for 10 minutes to separate serum. Separated serum is subdivided into 2 plain tubes and kept in deep freezer at -70 °C for determination of:
   1-Fasting serum insulin 2-Serum pentosidine.

Two hours after meal another blood sample was taken on fluoride-oxalate for measurement of 2h pp blood glucose.

Statistical analysis

All data were analyzed using **SPSS 15.0** for windows (SPSS Inc., Chicago, IL, USA) &**MedCalc 13** for windows (MedCalc Software bvba). Continuous variables were expressed as the mean ± SD. Continuous variables were checked for normality by using **Shapiro-Wilk test**. **Independent Student t-test** was used to compare normally distributed variables between two groups. **Mann Whitney U (MW) test** was used to compare non-normally distributed variables between two groups. **One way ANOVA** was used to compare normally distributed variables between three groups. **Kraskall Wallis H test** was used to compare non-normally distributed variables between three groups. Post-hoc LSD was used. Percent of categorical variables were compared using the Chi-**square (χ^2) test. Pearson's correlation analysis** wasdone between pentosidine, HOMA-IR and selected study parameters except for non-normally distributed parameters; **Spearman's rank correlation coefficient** was calculated. We consider (+) sign as indication for direct correleation i.e. increase frequency of independent lead to increase frequency of dependent & (-) sign as indication for inverse correleation i.e. increase

frequency of independent lead to decrease frequency of dependent, also we consider values near to 1 as strong correleation& values near 0 as weak correleation.

3. Results

Table 1: Comparison between the studied groups and sub groups as regard demographic data and anthropometric measurements:

Demographic data	Group A (N=33) Mean ± SD		Group B1 (N=16) Mean ± SD		Group B2 (N=17) Mean ± SD		Test	P
Age (years)	27.82 ± 4.92		26.13 ± 5.05		30.24 ± 6.97		F 2.312	0.107 (NS)
Sex	No	%	No	%	No	%	$\chi 2$ 1.733	0.420 (NS)
Male	20	60.6%	9	56.3%	7	41.2%		
Female	13	39.4%	7	43.8%	10	58.8%		
Smoking	12	36.3%	5	31.2%	3	17.6 %	$\chi 2$ 1.87	0.392 (NS)
BMI (Kg/m2)	23.48 ± 2.36		23.75± 2.97		26.41 ± 3.50 ab		F 6.369	0.003 (HS)
Waist circumference (cm)	87.97 ± 6.87		95 ± 9.35 a		99.41 ± 5.87 a		KW 22.167	<0.001 (HS)

Table (1) shows that:
a = significant difference in relation to group A
b= significant difference in relation to subgroup B1

- There is no significant difference between studied groups as regard age, sex and smoking status.
- There is significant difference among the studied groups as regard BMI and waist circumference.

Table 2: Comparison of the mean value ± SD of routine laboratory data among studied groups and subgroup:

Complete blood picture	Group A (N=33) Mean ± SD	Group B1 (N=16) Mean ± SD	Group B2 (N=17) Mean ± SD	Test	P
WBC ($x10^3/mm^3$)	6.57 ± 1.91	6.93 ± 2.08	7.17 ± 1.87	KW 1.243	0.537 (NS)
Hb (g/dl)	13.02 ± 1.16	13.30 ± 0.97	13.52 ± 0.82	F 1.367	0.262 (NS)
Platelet count ($x10^3/mm^3$)	301.06 ± 77.29	327.93 ± 91.08	324.11 ± 87.75	KW 3.667	0.213 (NS)
Total serum bilirubin (mg/dl)	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	KW 2.280	0.320 (NS)
Direct serum bilirubin (mg/dl)	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	F 0.992	0.377 (NS)
ALT (U/L)	26.9 ± 6.2	27.7 ± 6.5	28.06 ± 4.9	F 0.240	0.788 (NS)
AST (U/L)	24.7 ± 7.3	28.3 ± 7.6	29.4 ± 6.2	KW 5.958	0.051 (NS)
Total protein (g/dl)	7.4 ± 0.5	7.5 ± 0.5	7.3 ± 0.5	KW 1.091	0.580 (NS)
Albumin (g/dl)	4.0 ± 0.4	4.0 ± 0.4	4.2 ± 0.5	KW 2.219	0.330 (NS)
INR	0.9 ± 0.05	0.9 ± 0.07	0.9 ± 0.2	KW 1.076	0.584 (NS)
Serum creatinine (mg/dl)	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	F 0.448	0.641 (NS)
CRP (mg/L)	2.01 ± 0.62	2.17 ± 0.69	1.85 ± 0.56	F 1.069	0.350 (NS)

Table (2) shows that:

- There is no significant difference among studied groups as regard routine laboratory data.

Table 3: Comparison mean value ± SD of metabolic parameters and pentosidine among studied groups and subgroups:

Lipid profile	Group A (N=33)	Group B1 (N=16)	Group B2 (N=17)	Test	P
	Mean ± SD	Mean ± SD	Mean ± SD		
Cholesterol (mg/dl)	137.7 ± 19	137 ± 29.6	192 ± 62.9 ab	KW 11.253	0.004 (HS)
Triglycerides (mg/dl)	138 ± 16.6	137.5 ± 31.4	189.5 ± 54.2 ab	KW 12.545	0.002 (HS)
Fasting blood glucose (mg/dl)	93.6 ± 4.4	93.6 ± 4.6	118.7 ± 5.4 ab	KW 37.468	0.002 (HS)
2 hr postprandial blood glucose (mg/dl)	129.6 ± 6.8	130.7 ± 5.4	181 ± 10.6 ab	F 276.091	<0.001 (HS)
Fasting Insulin level (mIU/L)	5.1 ± 1.6	5.3 ± 1.8	8.4 ± 3.3 ab	KW 11.393	0.003 (HS)
HOMA-IR	1.17 ± 0.32	1.22 ± 0.37	2.46 ± 1.00 ab	F 29.822	<0.001 (HS)
Pentosidine level (ng/mL)	51.15 ± 11.2	55.9 ± 9.1	68.5 ± 7.8 ab	F 17.076	<0.001 (HS)

Table (3) shows that:

- There is significant difference among studied groups as regard cholesterol and triglycerides levels, fasting blood glucose, 2hour postprandial blood glucose, fasting serum insulin levels, HOMA-IR and serum pentosidine levels.

Table 4: Correlation coefficient between serum pentosidine (ng/ml) & selected study parameters in B1 and B2 subgroups

Variable	Group B1 (*n*=25)		Group B2 (*n*=25)	
	r	P	r	P
Age (years)	+0.132‡	0.626 (NS)	+0.376‡	0.137 (NS)
Smoker	-0.307	0.247 (NS)	-0.263	0.361 (NS)
BMI (Kg/m2)	+0.049‡	0.858 (NS)	-0.236‡	0.362 (NS)
Waist circumference (cm)	+0.063§	0.816 (NS)	-0.223‡	0.389 (NS)
Cholesterol (mg/dl)	-0.267§	0.318 (NS)	+0.430‡	0.085 (NS)
Triglycerides (mg/dl)	-0.255§	0.341 (NS)	+0.466‡	0.059 (NS)
FBG (mg/dl)	+0.268‡	0.315 (NS)	+0.139‡	0.596 (NS)
2 hr postprandial Blood Glucose (mg/dl)	+0.129‡	0.633 (NS)	-0.123‡	0.637 (NS)
Fasting Insulin level (mIU/L)	+0.177‡	0.513 (NS)	+0.588‡	**0.013 (S)**
HOMA-IR	+0.244‡	0.363 (NS)	+0.588‡	**0.013 (S)**

Table (4) shows that:

- There is significant positive correlation between serum pentosidine level and each of fasting insulin level and HOMA-IR in group B2.
- While there was no significant correlation between pentosidine and each of age, Smoking status, BMI, waist circumference, cholesterol, triglycerides, FBG and 2hr postprandial blood glucose in group B1and groupB2.

4. Discussion

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels[5].

Prolonged exposure to hyperglycaemia is now recognized as the primary causal factor in the majority of diabetic complications. There are several, well researched theories of how chronic hyperglycemia can lead to micro or macrovascular disease in diabetes including the advanced glycation end products (AGEs) theory[28].

AGEs constitutes a large group of heterogeneous molecules formed by the non enzymatic reactions of sugar with free amino groups of proteins, lipids, peptides and nucleic acids within the so-called "Maillard reaction", a tribute to the French scientist Louis Camille Maillard (1878–1936)[21].

pentosidine is one of the best chemically characterized AGEs in humans and acts as a marker for the formation and accumulation of AGEs[13].Pentosidine was characterized as a sensitive marker for all AGEs[8].

pentosidine levels were significantly increased in diabetic patients with retinopathy and in diabetic patients with nephropathy. Pentosidine levels were increased markedly with the severity of microangiopathy[19].

In this regard, we have done this study to find out any changes of the level of AGEs in offspring of type 2 diabetes with and without impaired glucose tolerance and its relation toeach of age, body mass index and insulin resistance in those subjects.

Our study show that there is significant increase in BMI and waist circumference in offspring of type 2 diabetes with impaired glucose tolerance in relation to healthy subjects and offspring with normal glucose tolerance, this was consistent with the results of ***Shengxu et al.(2014)***[29] who reported that for each SD increase in BMI (2.1 kg/m^2) and waist circumference (8.3 cm), fasting glucose levels increased by

0.128 and 0.170 mmol/L in men, and by 0.112 and 0.167 mmol/L in women, respectively. The corresponding increases for 2-hour post load glucose levels were 0.121 and 0.217 mmol/L in men and 0.241 and 0.362 mmol/L in women. When simultaneously included in the same model, these associations with waist circumference were stronger than with BMI.

Also ***Díaz-Redondo et al.(2015)***[11]reported that both general and, more specifically, abdominal obesity were associated with the presence of prediabetes.General obesity was defined as a Body Mass Index (BMI) ≥30 Kg/m2 and abdominal obesity as a waist circumference ≥102 cm in men and ≥88 cm in women[27].

Moreover, ***Bardenheier et al.* (2013)**[7] reported that when a wide number of risk factors of prediabetes had been examined simultaneously, waist circumference had showed the strongest direct effect on prediabetes**.** Although the mechanisms are not clear, excess adipose tissue has been reported to release fatty acids that induce resistance to insulin in the muscle and in turn leads to elevation of plasma glucose levels [13]**.** Also, measures of abdominal obesity have been found as the strongest predictors of diabetes[33]**.**

Our study show that there significant increase in cholesterol and triglycerides levels in offspring of type 2 diabetes subjects with impaired glucose tolerance in relation to healthy subjects and offspring with normal glucose tolerance. This was in agreement with ***Love-osborne et al.(2006)***[20]who states that a fasting triglyceride level ≥150 mg/dL was strongly associated with IGT and may help to identify at risk adolescents who should undergo formal glucose tolerance testing.

Also, in the **Botnia study** (4483 men and women aged 35–70 years; 1697 with diabetes and 798 IGT), the prevalence of elevated plasma triglycerides [151 mg/dL] was up to two times higher in those with IGT compared with those with normal glucose tolerance as reported by ***Isomaa et al. (2001)***[16].

In the current study, a significant increase in fasting blood insulin and HOMA-IR was increased in the impaired glucose tolerant offspring group as compared to control and normal tolerant offspring group, these findings cope with ***Johnson et al.(2010)***[18]who reported that fasting insulin levels is increased in patients with impaired glucose tolerance and this will increase the likelihood of identifying prediabetes, also it was reported that increased HOMA-IR index predicted prediabetes.

The current study show that there is significant increase in serum pentosidine levels in offspring of type 2 diabetes subject with impaired glucose tolerance in relation to healthy subjects and offspring with normal glucose tolerance, this was in agreement with ***Monden et al., (2013)***[23]which could be explained by the fact that AGEs is increased in insulin resistance due to its role in adipose hypertrophy and subsequent effect on insulin sensitivity.

On the other hand, ***Chakarova et al. (2013)***[10]have reported that no significant difference in AGEs accumulation could be detected in subjects with prediabetes in comparison to normal glucose tolerance subjects.In***Chakarova et al*** study tissue AGEs accumulation was assessed non invasively measuring the skin fluorescence of ultra violet light on the ventral side of the lower arm.In the past, measurement of fluorescence intensity has primarily involved estimating the AGEs content, which is arelatively simple process. However, a number of concerns have been raised regarding estimation of AGEs in vivo simply by measuring fluorescence intensity, given the great number of AGEs which do not fluoresce and the fact that some non-AGE compounds show similar fluorescence properties to AGEs, potentially confounding results as reported by***Nagai et al. (2010)***[24]**.**

The current study show that there is significant positive correlation between pentosidine level and fasting blood insulin and HOMA IR in impaired glucose tolerance group, this was in line with***Monden et al., (2013)***[23] who investigated role of AGEs in metabolic syndrome and found that AGEs affect adipocyte hypertrophy and insulin sensitivity.

Also Uribarri et al. (2007)[31]found an association between AGEs and the homeostasis model assessment (HOMA IR) levels of normal persons which could be linked to metabolic processes, which may precede insulin resistance, diabetes mellitus, or vascular dysfunction at any age.

Cai et al. (2012)[9]found that oral AGEs promote insulin resistance and diabetes by depleting the antioxidant defenses of AGER-1 and sirtuin.

Our study showed that there was no significant correlation between serum pentosidine and age which was against the results obtained by***Chakarova et al. (2013)***[10] this may came from the high mean of the age of the subjects included in ***Chakarova*** study which was (51.6±11, 6) in relation to the mean of the age of the subjects included in our study which was (30.24 ± 6.97).***Uribarri et al.(2007)***[31] investigated whether AGEs intake correlated with glycotoxin levels, markers of inflammation and oxidative stress (OS) comparing older versus younger healthy adults. They studied 172 healthy volunteers in two groups (18–45 years) and (60–80 years). AGES were higher in the older group.

Our study shows that there was no significant correlation between serum pentosidine level and BMI in both normal and impaired glucose tolerant offspring of type 2 diabetes. This was against the results obtained by ***Chakarova et al. (2013)***[10] which stated that there was positive correlation between AGEs and BMI. This may came from the high mean of the BMI of the subjects included in Chakarova study which was (30.4±5.2kg/m^2) in relation to the mean of the BMI of the subjects included in our study which was (23.75 ± 2.97 kg/m^2) and (26.41 ± 3.50 kg/m^2) for normal and impaired glucose tolerant offspring respectively. It may also come from the relatively small number of subjects included in our study which was 66 in relation to the number of subjects included in ***Chakarova*** study which was185.

We can conclude that the high level of AGEs in offspring of type2 diabetes with impaired glucose tolerance due to hyperinsulinaemia may lead to many vascular complications

in pre diabetec state. Therefore we recommend screening offspring of type 2 diabetes for AGEs and early management to guard against vascular complicatios that may occur in pre diabetec state.

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