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ADVANCED GLYCATION END PRODUCTS MEASURED BY AGE READER IN A GROUP OF PATIENTS WITH OBESITY

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Abstract

Background and Aim: *Skin autofluorescence (AF) is a method used to detect the accumulation of Advanced Glycation End Products (AGEs) in skin collagen using AGE Reader. AGEs accumulation is favorised by chronic hyperglycemia and oxidative stress and these products may contribute to the pathogenesis of cardiovascular disease.* ***Materials and Methods****. The inclusion criteria for the study were: patients with Body Mass Index (BMI) ≥30kg/m². We excluded from the study patients of black color, because of the principle of fluorescence of AGE Reader, and also patients with diabetes. Patients underwent Oral Glucose Tolerance Test (OGTT) with 75g of glucose: fasting plasma glucose (FPG), two hours post glucose load, and HbA1c blood samples were analysed. Finnish diabetes risk score for predicting the incidence of diabetes (FINDRISK score) was calculated.* ***Results.*** *BMI had a negative correlation with AF (p<0.005), but Abdominal Circumference (AC) was not correlated with AF (p=0.065). No correlations were found between BMI and FPG, or blood sugar level two hour post glucose load. Glucose level at two hours post glucose load did not correlate with the AF.* ***Conclusion****. Our results do not confirm the theoretical assumption according to which AC and BMI, as markers of insulin resistance and metabolic disorders, are associated with the increase in AGEs, or the assumption that postprandial blood glucose levels would have a more important role in the development of chronic complications that AGEs could be pathogenic link. AF is not influenced by short-term variations in blood glucose such as blood sugar level at two hours post glucose load, but it is influenced by FPG and prolonged term variation in blood glucose as HbA1c.*

key words: *advanced glycation end-product (AGEs), obesity, AGE Reader*

Introduction

About AGEs: in 1912 a French chemist, Louis-Camille Maillard, performed a simple laboratory experiment that turned out to be a shortcut that created meat flavor and aroma by heating sugar and amino acids; this reaction has since been named „Maillard Reaction" or

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„Browning Reaction". The term AGEs was introduced to describe the end products that Maillard discovered that are produced in living organisms in normal physiological conditions. AGEs are the result of chemical chain reactions after an initial glycation reaction takes place. Schiff Base, Amadori, and Maillard products are the names given to the intermediate products according to the reserchers that discovered them, but not all literature uses the same term. Moreover, the literature is inconsistent about Maillard reaction products that are sometimes considered intermediates and sometimes end products. In this process there are generated different agents, which have oxidizing properties (hydrogen peroxide), or not (beta amyloid proteins that confer protection against oxidative stress) [1].

Role of AGEs: AGEs play an important role in the progression and development of chronic age-related diseases such as diabetes, chronic kidney disease and cardiovascular disease. Moreover, the level of AGEs in long-lived tissues serves as a memory of glycometabolic and oxidative stress and it is an important predictor for cardiovascular complications [2].

Measuring AGEs: until now, clinicians found it difficult to measure AGEs in their patients because of expensive, time consuming, poorly reproducible, lacking specificity and/or invasive methods. „AGE Reader" is a simple, non-invasive solution which allows clinicians to determine the AGE level within 30 seconds. Many AGEs have a characteristic fluorescence and researchers observed an association between tissue biopsies fluorescence and chronic complications [3]. AGEs are normaly produced in living organism under physiological conditions and the quantity increases with aging. Due to the selective absorption by skin compounds, subjects with darker skin color typically have lower AF values than subjects with white skin [4].

Objectives

The aim of our study was to to investigate whether obesity (evaluated by BMI, AC) corelates with AGEs; another objective was to see if AF is influenced by short-term variations in blood glucose (fasting plasma glucose and glucose at 2 hours post glucose load) and long term variation in blood glucose (HbA1c). The FINDRISK score shows the risk to become diabetic and we investigated wether it is correlated with AF. Most researches has linked smoking and age with the increase of AGEs and we searched also these correlations.

Materials and Methods

The study was conducted at Jean Verdier Hospital in Paris, Department of Endocrinology, on a group of 88 patients, 76 females and 12 males, aged between 19 and 68 years old, with a mean age of 41,36 years. The inclusion criteria for the study was: patients with BMI $\geq$30kg/m^2. There were excluded from the study patients with diabetes and also patients of black color, because of the principle of fluorescence of AGE Reader, that has a light source which illuminates the tissue of interest. This light excites fluorescent moieties in the tissue which will emit light with a different wavelength, and the major contribution in fluorescence comes from fluorescent AGEs. If the skin is dark, the emitted light will not pas and it can not be detected by the spectrometer. AGE Reader can be used on a skin scale from I-III after the

classification Thomas B. Fitzpatrick. The Fitzpatrick Scale is a numerical classification schema for the skin colour and it was developed in 1975 by Thomas B. Fitzpatrick, a Harvard dermatologist, in order to classify the response of different types of skin to UV light. OGTT with 75g of glucose was performed, blood samples for fasting plasma glucose and then two hours post glucose load were taken.

The Fitzpatrick Scale:

Type I (scores 0-7) White; very fair; freckles; typical albino skin. Always burns, never tans

Type II (scores 8-16) White; fair. Usually burns, tans with difficulty

Type III (scores 17-25) Beige; very common. Sometimes mild burn, gradually tans to a light brown

Type IV (scores 25-30) Beige with a brown tint; typical Mediterranean Caucasian skin. Rarely burns, tans with ease to a moderate brown

Type V (scores over 30) Dark brown. Very rarely burns, tans very easily

Type VI Black [5].

Statistical analysis. All calculations were performed using the Statistical Pakage for Social Sciences Software (SPSS) version 17.

Results

The statistical analysis found that BMI was negatively correlated with AF ($p<0.005$), (Figure 1), (Table 1, 2), but AC did not correlate with AF ($p=0.065$) (Figure 2). The mean for AF was 2.389, the lowest walue was 1.4 and the highest was 3.4. For BMI the mean value was 38.9 kg / m², the lowest value was 30.18 kg / m² and the highest was 59.6 kg / m².

Table 1. Descriptive statistics for BMI and AF.

	Mean	Std. Deviation	N
AF	2.389	.4134	88
IMC	38.9501	6.09735	88

Table 2. Correlation between BMI and AF (p = 0,01)

		AF	IMC
AF	Pearson Correlation	1	-.275**
	Sig. (2-tailed)		**.010**
	N	88	88
IMC	Pearson Correlation	-.275**	1
	Sig. (2-tailed)	**.010**	
	N	88	88

**Correlation is significant at the 0.01 level (2-tailed).

Pearson correlation value is quite small, -0.275, but it indicates a negative correlation between variables. The result is statistically significant with a p-value of 0.01 (Fig.)

The same is true for the other two correlation coefficients (nonparametrics), Kendall and Spearman respectively with values -0.185 and -0.266.

In the group of patients with Imparing Fasting Glucose (IFG), AF values are significantly different from patients without IFG, Sig = 0.017 by ANOVA-test; there is a statistical difference between the two groups, there is a clear influence of IFG on the AF, which has an average value of 2.62 vs an average value of 2.34 in patients without IFG (Figure 3).

Two hours post-load glucose levels did not correlate with the AF index, and no significant differences were observed between the patientis with Impaired Glucose Tolerance (IGT) and patients with normal glucose tolerance (p=0.439) (Figure 4).

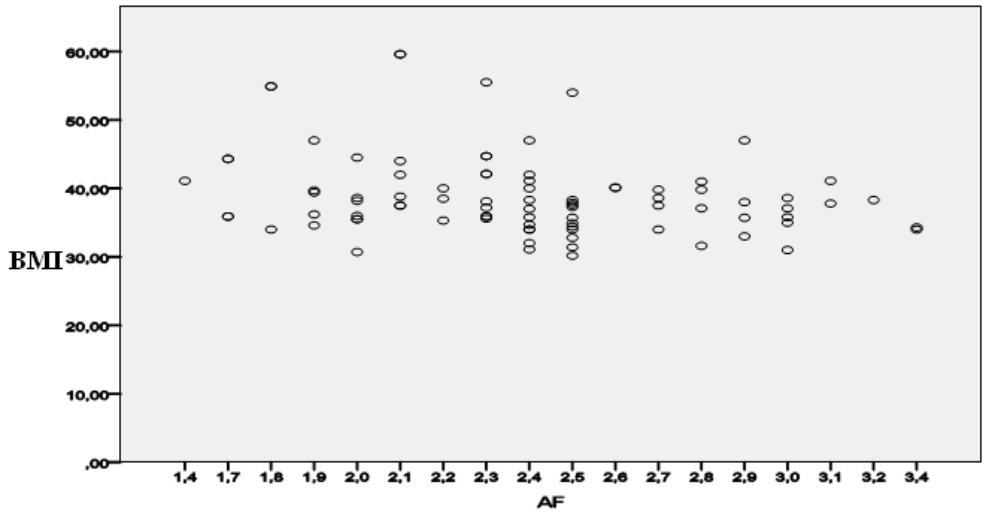

Figure 1. Correlation between BMI and AF (p <0.005).

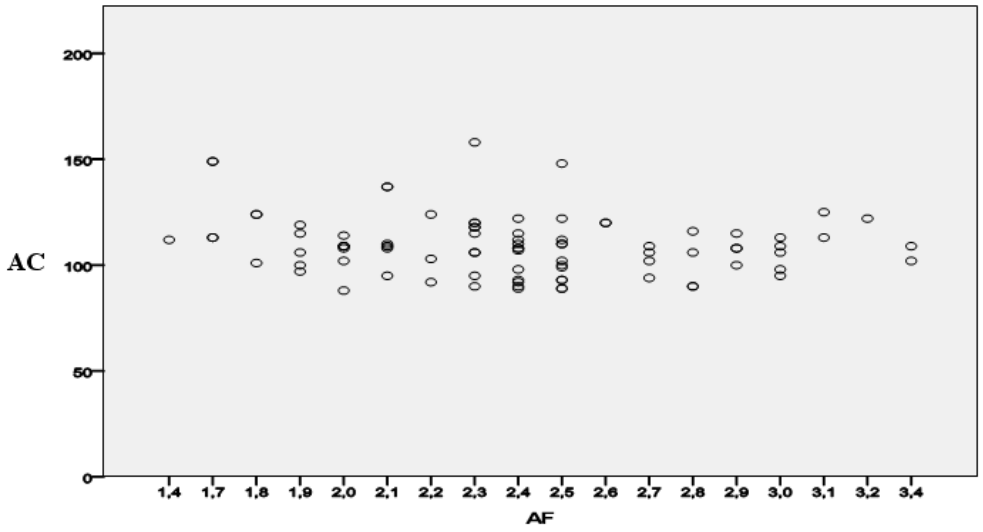

Figure 2. Correlation between AC and AF (p = 0.065).

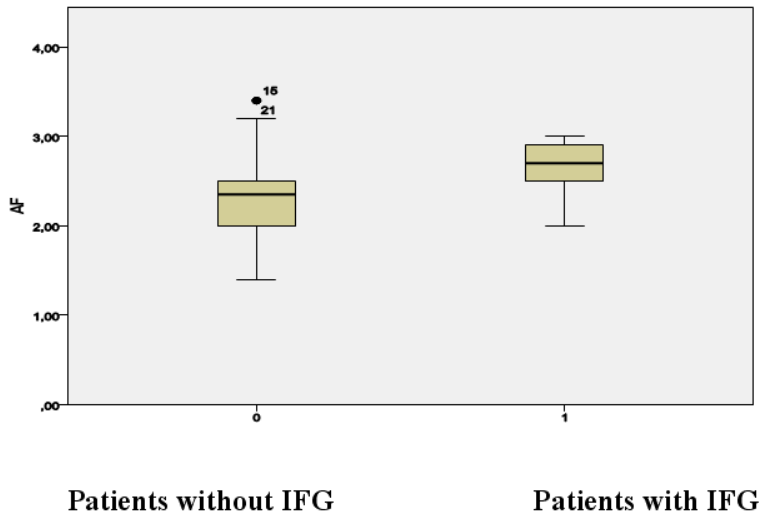

Figure 3. Corelation between AF and fasting glycemia (p = 0.017).

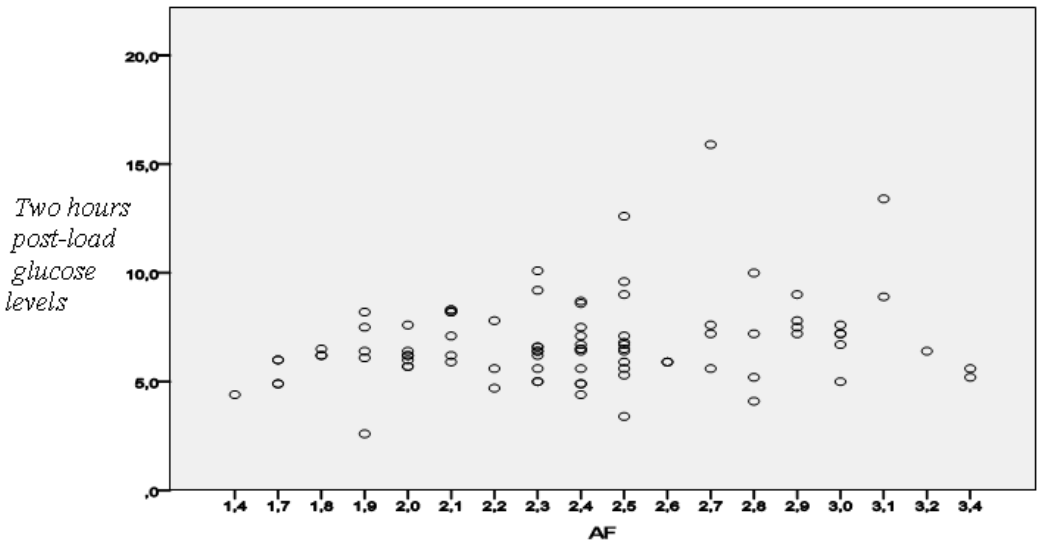

Figure 4. Correlation between two hours post-load glucose levels and AF index (p = 0.439).

Long term variation in blood glucose, defined by HbA1c, correlated with AF (p=0,00) (Figure 5), and also with FINDRISK score, that shows that the risk of development of diabetes correlated with AF (p=0,039) (Figure 6). FINDRISK score finds subjects with increased frequency of unhealthy lifestyle parameters and it is used to identify subjects at high risk for type 2 diabetes assessing age, waist circumference, BMI, medication against high blood pressure, history of high blood glucose, physical activity and daily consumption of vegetables/fruits [6].

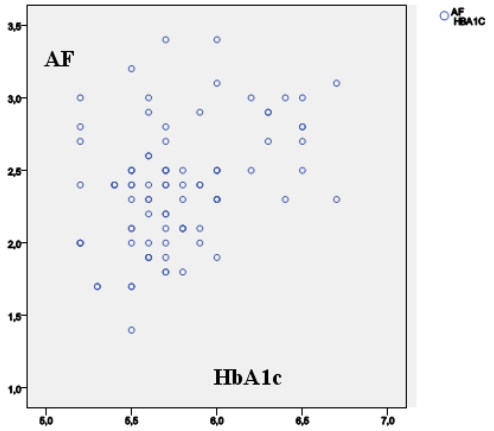

Figure 5. Correlation between HbA1c and AF (p = 0.00).

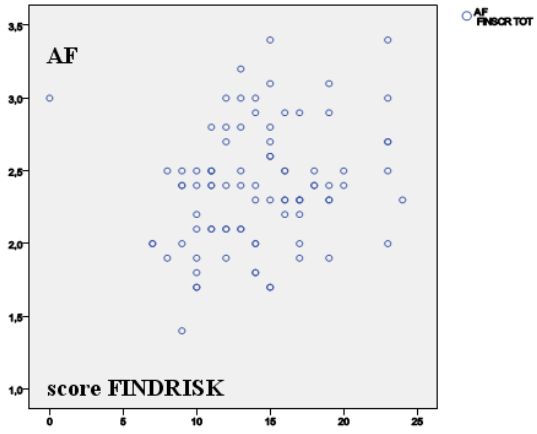

Figure 6. Correlation between score FINDRISK and AF (p = 0.039).

Although most researches have linked the increase of AGEs with smoking and also with age, in our group we did not found any connection, but statistical data are not so relevant because only 21 subjects are smokers (Figure 7, 8).

Discussion

AGEs accumulate over a person's lifetime, but this process occurs more rapidly in certain pathologic conditions such as diabetes mellitus (hyperglycemia causes oxidative stress), chronic kidney disease

and/or cardiovascular disease; therefore AGEs formation can be increased beyond normal levels in this patients [7]. Accumulation of AGEs is an important factor in the development of chronic complications of these conditions. AGEs may be formed exogenously by heating (cooking) or endogenously through normal metabolism and aging. It is considered that AGEs play a role as proinflammatory mediators in gestational diabetes as well [8]. In diabetic patients, AGEs correlate closely with early kidney, eye and nerve disease. Moreover, they are good predictors of cardiovascular morbidity and mortality. New drugs, aimed at preventing formation of AGEs or breaking AGEs are currently in trial [9].

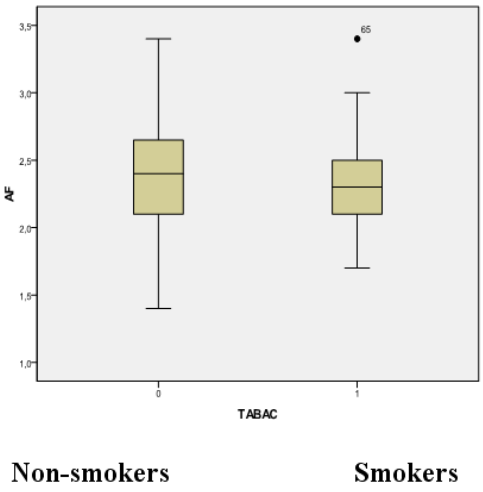

Figure 7. Box plot graph AF, Smoking

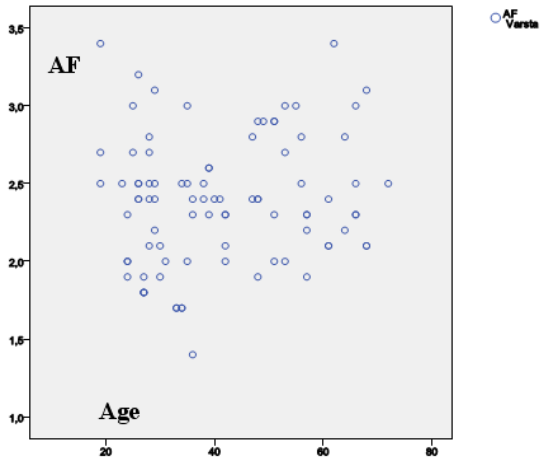

Figure 8. Correlation between AF and Age (p = 0.243)

The mean reference values of AF in healthy Caucasian population is 2.3 (0.6) and according to different age groups: 40-49 years: 1.8 (0.4); 50-59 years: 2.1 (0.3); 60-69 years: 2.5 (0.6); 70-79 years: 2.8 (0.5) ; ≥80: 2.9 (0.5) [10]. In our population the mean reference of AF was 2.389.

Yue X and colab. conducted a study in healthy Chinese individuals in order to establish the values of AF by the AGE Reader; they also took into account age, gender, skin reflectance, smoking habits and alcohol consumption. According to them, the mean reference values of AF are: 20-29 years: 1.54-1.62 arbitrary units; 30-39 years: 1.66-1.75; 40-49 years: 1.78-1.89; 50-59 years: 1.87-2.03; 60-69 years: 1.86-2.09; 70-79 years: 1.97-2.31. The value of AF was strongly related to age, but no significant difference between males and females was found. AF was lower in non-smokers than in smokers [11]. In persons with low skin reflectance (<10%), skin AF was dependent on skin colour, but was still related to age [12, 13]. In our group, we did not find any connection of AGEs with smoking and also with age.

In smokers, tobacco-derived AGEs accumulate on plasma low density lipoprotein (LDL), structural proteins within the vascular wall, and in the crystalline lens' proteins. These data point to a new source of AGEs products in the human environment, enlights the role of Maillard chemistry in pathological processes and provide new hypothesis for atherosclerosis pathogenesis and other diseases related to smoking [14, 15].

In the study performed on 21 subjects, skin AF was examined with the AGE Reader before and after a high fat meal with 594 kcal, with amount of AGEs considered medium. Skin AF increased postprandially in individuals with diabetes mellitus by 11.6% and in healthy subjects by 8.7% [16].

It is known that central obesity enhances oxidative stress, therefore people with central obesity might already have increased accumulation of AGEs before the onset of diabetes or cardiovascular disease. To confirm this hypothesis, scientists compared the distribution of skin AF and its association with clinical and biochemical parameters in subjects with and without central obesity; the conclusion was that the relationship between AC and AF is not as strong as previously thought. We found in literature few studies addressing this issue, but all consistent with our results. In the study accomplished by Engelsen Corine and colab. in Holland on a population of 861 subjects with abdominal obesity and 431 without abdominal obesity, the conclusion was that the relationship between AC and AF is not as obvious as previously thought. Lutgers et al. were the only ones to search the relationship between obesity and skin AF in a population without established diabetes or cardiovascular disease. In their multivariable analysis BMI, as measure of obesity, did not show a significant association with skin AF [13]. Another study accomplished by Noordzij MJ, Lefrandt JD, Graaff R, Smit MAJ in the Netherlands led to the conclusion that acute changes during glucose OGTT, conducted in 56 people, with varying degrees of glucose tolerance, did not affect AF [17].

Conclusion

Our results do not confirm the theoretical assumption according to which AC and BMI, as markers of insulin resistance and metabolic disorders are associated with the increase of AGEs: in our study BMI had a negative correlation with AF and AC did not correlate with AF. Alhough our results do not confirm the assumption that postprandial hyperglycemia has a more important role than basal glycemia in the development of chronic complications, nevertheless AGEs are considered to have a pathogenetic role in their appearance.

There is a clear influence of IFG on the AF, but two hours post glucose load glycemia did not correlate with the AF index and no significant differences were observed between the patients with IGT and patients with normal glucose tolerance. AF is not influenced by short-term variations of blood glucose.

HbA1c correlated with AF; FINDRISK score, that shows the risk of development of diabetes correlated with AF.

Although most researches have linked aging and smoking with the increase of AGEs, in our study we did not find any connection, but statistical data are not so relevant probably because only a quarter of subjects are smokers. Limitations of the AGE Reader as a measure of AGEs accumulation are: not all AGEs exhibit fluorescent properties and therefore they can not be quantified with the AGE Reader. Other interstitial, cellular and vascular components can be also fluorescent [18].

Larger studies are needed to assess the risk of obese patients to develop chronic complications because of the accumulation of AGEs in tissues and to develop effective means of prevention therapy.

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