Chapter 6

Glucose Tolerance and Elders – Lessons We Have Learned and Challenges for the Future

Sylwia Dzięgielewska-Gęsiak and Ewa Wysocka

Additional information is available at the end of the chapter

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1. Introduction

The worsening of glucose metabolism as well as increasing risk for cardiovascular disease while aging is documented (Wild S. et al., 2004; Okereke OI. et al., 2008). According to gold standard research in diabetes - the United Kingdom Prospective Diabetes Study (UKPDS) (Matthews D.R 1999; Turner R.C. et al., 1998) - about 40% of patients with type 2 diabetes mellitus (T2DM) suffer from late diabetic complications, including atherosclerosis, hypertension and dyslipidemia and microangiopathies (nephropathy, retinopathy). From epidemiological point of view, 40% of dysglycemic patients are 65 years old or over (Barnett T. 1998; Harris M. 19962).

An impaired fasting glycemia (IFG) and an impaired glucose tolerance (IGT) are categories of increased risk for type 2 diabetes mellitus (prediabetes) (ADA 2012). There is no separate algorithm for dysglycaemia in elders, however glucose concentrations in plasma increases with aging (from 30 years old, fasting glycemia about 1-2 mg/dl for the every decade and postprandial glycaemia about 2-4 mg/dl for the every decade), and is caused by insulin-resistance (Winger JM & Hornick T. 1996). Paralleled to glucose concentration glycated hemoglobin - HbA_{1c} rise with age – round 0,11 to 0,15 % of HbA_{1c} per decade (Nuttal F.Q. 1999).

Aging is a universal process that can be determined by genetic, the environment or diseases. Biological theories are the most promising in relation to finding answers about aging.

1.1. Aging theories

There are many aging theories. From the very easy one, like programmed death theories, to the more complicated as concerning mistake accumulation theories (Table 1).

THEORIES OF PROGRAMED DEATH	THEORIES OF MISTAKE ACCUMULATION
programmed cellular aging theory clock theory	autoimmune theory random mistake theory free radical theory cross-links theory calories restriction theory glycation theory

Table 1. Theories of aging (examples)

The gerontological theories try to explain aging from the biological point of view, and explain that not only still dividing cells but also cells that are unable to divide, both can be targeted by aging (Kirkwood TBL. & Austad SN. 2000).

1.1.1. Free radical theory

The free radicals theory postulates that the aging process is the initiation of free radical reactions (Harman D. 2003) and in hyperglycemic patients may rise due to increased free radicals production. The theory suggests that most of free radicals are initiated and produced by mitochondria and a life span is determined by the rate of free radical damage to the mitochondria (Harman D. 1983).

1.1.2. Glycation theory

The theory is based on non-enzymatic reaction - glycation. The theory suggests that glucose acts a mediator of aging. The cross-links between glucose and proteins lead to stable intra- and inter-molecular changes which can influence cells, tissues and organs (Soškić V. et al., 2008). Thus the glycation may have tremendous cumulative effect during a person's life leading to shorter life span in diabetics.

1.1.3. Caloric restriction

The theory proposes that life span can be prolonged by lover calorie intake but the compounds are the full qualitative (Vitetta L. & Anton B. 2007). Decreases in calorie intake reduce free radicals production – lower oxidative stress and decreased inflammatory processes (Ungvari Z. et al., 2008).

Patients with hyperglycemia may present more than one reason of increased aging process.

1.2. Late complications of hyperglycemia

As hyperglycemia starts (prediabetes, diabetes mellitus) late metabolic complications, caused by glucose toxicity (Robertson RP. & Harmon JS. 2006), oxidative stress (Cumaoğlu

A. et al., 2010), dyslipidemia (Gordon L. et al., 2010), increased polyol pathway (Obrosova I.G. 2005) and glycation of protein (Xie X. et a., 2010), increase.

1.3. Protein glycation

Protein glycation and oxidative stress in a context of aging is widely discussed (Robertson RP. 2004; Muller F.L. et al., 2007; Soškić V., et al. 2008). Protein glycation depends on duration of hyperglycemia and increase in aging and co-morbidities (Peppa M. & Vlassara H. 2005).

Non-enzymatic glycation of proteins leads trough Amadori products such as glycated albumine (fructosamine), glycated hemoglobine (HbA_{1c}) to Advanced Glycation End Products (AGEs) eg. N^{ε}-carboxymethyllysine (CML) (figure 1), bio-active molecules which accumulate with age. AGEs are produced in human as a part of normal metabolism, but are elevated in diabetic patients, and depends on hyperglycemia duration and level (higher and longer hyperglycemia leads to higher AGEs concentration).

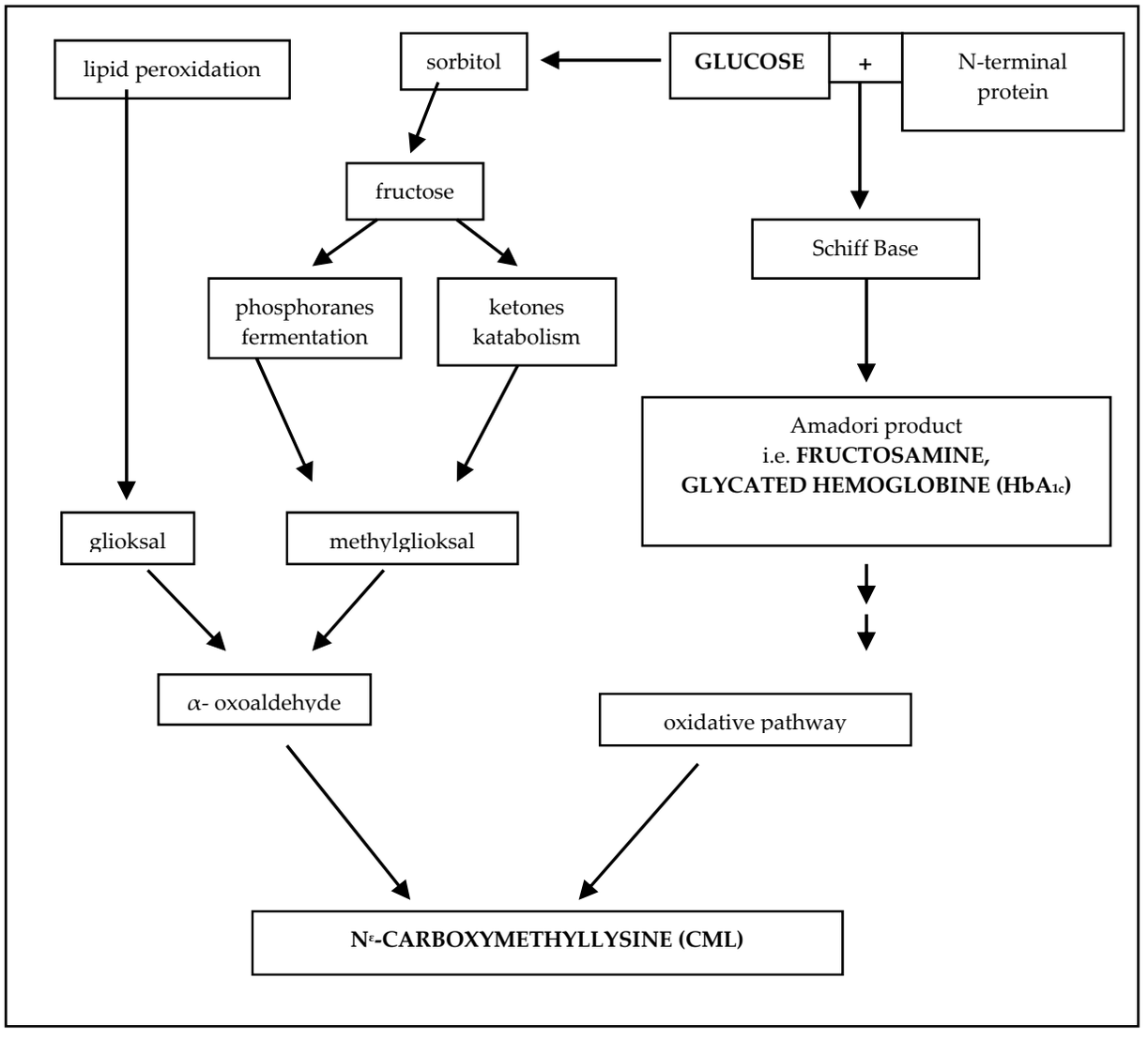

Figure 1. Pathways for the Advanced End Products Formation i.e. N^{ε}-carboxymethyllysine.

1.3.1. Fructosamine

Fructosamine is an Amadori product that is formed as a result of lysine side chain of protein modification by glucose and its serum level is reported to increase in diabetes. The product serves as short term marker (2-3 weeks) of glycemic control (Armbruster DA.1987; True MW. 2009). Fructosamine can undergo oxidative cleavage leading to the formation of Avanced Glycation End products (AGEs), which are implicated in long-term complications of diabetes mellitus.

1.3.2. Glycated hemoglobin, HbA_{1c}

Glycated hemoglobin, HbA_{1c}, is an Amadori product that is now recognized as a marker of glycemic control. Glycated hemoglobine, HbA_{1c} measures blood glucose control over a period of eight to twelve weeks (Rohlfing CL. et al., 2002). The results from different trials (The ACCORD trial (ACCORD Group 2008), the Veterans Affairs Diabetes Trial (VADT Investigators 2009), the Action in Diabetes and Vascular Disease—PreterAx and DiamicroN Modified Release Controlled Evaluation (ADVANCE) trial (ADVANCE Collaborative Group 2009)), have created a debate about the optimal choice of glycated hemoglobine target. The International Diabetes Federation recommends a level of less than 6.5% (IDF 2006) whereas the American Diabetes Association recommends an HbA_{1c} level of less than 7.0% as the standard of glycemic treatment goal (ADA 2010). the American Diabetes Association recommends a HbA_{1c} level of less than 8.0% as the glycemic treatment goal for the elderly diabetic persons. Nowadays the HbA_{1c} is also recommended for diagnosis of diabetes (ADA 2010).

1.3.3. Advanced glycation end products - N^{ε}-carboxymethyllysine, CML

N^{ε}-carboxymethyllysine, {(2S)-2-amino-6-(carboxymethylamino)hexanoic acid}, (CML), (figure 2) one of dominant Advanced Glycation End products (AGEs), is formed in vivo by oxidative cleavage of the Amadori product threulosyl-lysine (combined non-enzymatic glycation and glycoxidation) and of metal-catalyzed oxidation of LDL or peroxidation of polyunsaturated fatty acids in the presence of fructose-lysine (Fu M.X. et al., 1996) (As in figure 1). Thus CML may serve as bio-marker of oxidative stress and protein damage in aging, diabetes mellitus and atherosclerosis (Southern L. et al., 2007; Semba R. D. et al., 2009). Excessive formation of CML has been proposed to be an important mechanism for accelerated atherogenesis in patients with hyperglycemia.

There are many evidences indicating that protein glycation and oxidative stress may accompany and explain metabolic late diabetic complications. Investigation of glycated proteins (fructosamine, HbA_{1c}) and AGEs (i.e. CML), in early stages of glucose metabolism problems, may explain metabolic complications in hyperglycemic elderly persons.

The aim of the study was to analyze fructosamine in plasma, HbA_{1c} in blood and CML serum concentrations among elderly patients with increased risk for type 2 diabetes mellitus (prediabetes) and normal glucose tolerance.

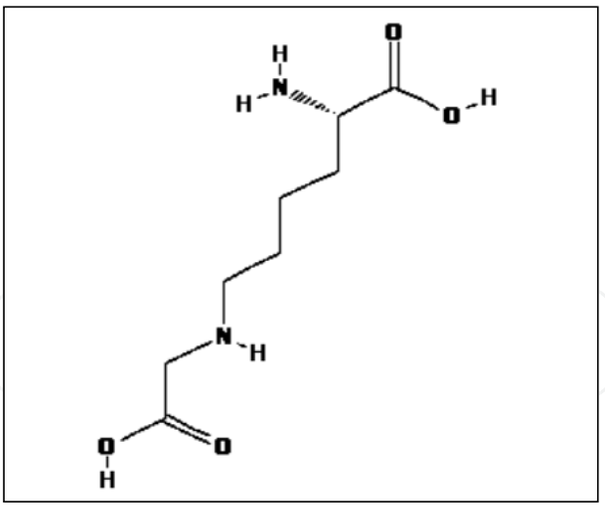

Figure 2. Chemical structure of Nε-carboxymethyllysine, (2S)-2-amino-6-(carboxymethylamino)hexanoic acid, (CML) – one of the Advanced Glycation End product.

2. Material and methods

The study was performed in the Department of Clinical Biochemistry and Laboratory Medicine, Chair of Chemistry and Clinical Biochemistry of Poznan University of Medical Sciences under the permission from local ethics group in accordance with the Declaration of Helsinki of 1975 for Human Research and the study protocol was approved by the Bioethics Committee of Poznan University of Medical Sciences in Poznan, Poland.

Subjects and Settings: This study population consisted of 313 elderly Caucasians (65 and older) from Poznan metropolitan area, and after following exclusion criteria (below), 58 of them with no acute diseases or severe chronic disorder were enrolled to the study. They were performed complete physical examination including the measurement of waist circumference (WC) in centimeters, percentage of body fat (FAT) by bioimpedance using BodyStat equipment and the calculation of body mass index (BMI=kg/m^2). Arterial blood pressure: systolic (SBP) and diastolic (DBP) were measured twice by sphyngomanometer MEDEL PALM PRO No: 91431 (Novamedica Ltd, Birmingham, United Kingdom) in sitting position, after at least 5 minutes rest. The arterial blood pressure was expressed than as mean value.

The exclusion criteria were the presence of following conditions: a coronary artery disease, a history of diabetes, the neoplastic diseases, the inflammatory diseases, a previous therapy, use of anti- and oxidant drugs, alcohol use, smoking and electrocardiography findings specific for myocardial ischaemia/infarct.

Additional biochemical exclusion criteria were microalbuminuria and macroalbuminuria as albumin/creatinin ratio > 30 mg of albumin/1 g of creatinin in fresh morning urine sample and decreased estimated Glomerular Filtration Rate (eGFR) less than 60 ml/min/m^2 based on Modified Diet in Renal Diseases (MDRD) formula:

$$\text{eGFR (ml/min/1.75m}^2\text{)} =$$
$$= \{186 \times [\text{creatynin}]^{-1,154} \times [\text{age}]^{-0,203} \times 0{,}742 \text{ [for women]} \times 1{,}210 \text{ [for Afroamerican]}\}$$

Blood sampling and biochemical analysis: The studied subjects were collected ulnar venous blood twice: at 0 minute (fasting) and at 120 minute of the 75,0-g OGTT. Fasting plasma samples without hemolysis were used for glucose and lipid determinations. Fasting serum samples designated for fructosamine and N^{ε}-carboxymethyllysine measurements were frozen and stored at –25° C until assayed, separately. Blood collected at 120 minute of OGTT was used for plasma glucose determination.

Glucose and lipids assay. Oral glucose tolerance test (OGTT) was performed according to WHO recommendations (WHO 1999) between 7.00-9.00 am. Glucose concentration was determined at 0 min (fasting) and 120 min (postprandial) following a standard dose of 75 g glucose load. Glucose and lipid parameters including total cholesterol (T-C), high density lipoproteins cholesterol (HDL-C), triacyloglycerydes (TG) concentrations were evaluated by enzymatic methods using bioMerieux reagent kit (Marcy-l'Etoile, France) and the UV-160A Shimadzu spectrophotometer (Shimadzu Co., Kyoto, Japan). Low density lipoproteins cholesterol (LDL-C) was calculated using the Friedewald formula:

$$\text{LDL-C [mmol/l]}=\{[(\text{T-C})-(\text{HDL-C})]-(\text{TG}/2{,}2)\}$$

The reference sera: RANDOX Assayed Human Multi Sera Level 1 (as normal) and RANDOX Assayed Human Multi Sera Level 2 (as pathological) (Randox, Crumlin, United Kingdom) were used for monitoring the accuracy of the determinations.

Results of OGTT allowed to classify subjects for normal glucose tolerance (NGT) (n=30, mean age 69,5 years) and prediabetic (PRE) (n=20, mean age 70,0 years) categories, while newly diagnosed type 2 diabetes mellitus (T2DM) patients were excluded from the study. The interpretation of oral glucose tolerance test is presented in Table 2 (ADA 2012).

Categories of glycemia during OGTT		Plasma glucose concentration	
		Fasting (at 0 min.)	at 120 min.
Normal glucose tolerance (NGT)		< 5,6 mmol/l < 100 mg/dl	< 7,8 mmol/l < 140 mg/dl
High risk of diabetes (Prediabetes, PRE)	Impaired Fasting Glycemia (IFG)	5,6 – 6,9 mmol/l 100 – 125 mg/dl	< 7,8 mmol/l < 140 mg/dl
	Impaired Glucose Tolerance (IGT)	< 7,0 mmol/l < 126 mg/dl	7,8 – 11,0 mmol/l 140 –199 mg/dl
Diabetes mellitus (DM)		< 7,0 mmol/l < 126 mg/dl	≥ 11,1 mmol/l ≥ 200 mg/dl

Table 2. The interpretation of oral glucose tolerance test (OGTT)

Fructosamine assay. Glycated albumine (fructosamine) was determined using calorymetric metod based on the ability of ketoamines to reduce nitrotetrazolium-blue (NBT) to formazan in an alkaline solution. The rate of formation of formazan is directly proportional to the concentration of fructosamine. The measurement was done by Cobas 400 (Roche Diagnostics, Mannheim, Germany).

The sensitivity of this assay was 0,14 μmol/l with intra-assay coefficient of variation (CV) and inter-assay CV precision at 2,8% and 0,65% respectively.

HbA1c assay. Glycated hemoglobin, (HbA_{1c}) was determined using ion exchange high performance liquid chromatography (HPLC), D-10 (BioRad, Heidelberg, Germany). Using a specific standardized measurement set established through the National Glycohemoglobin Standardization Program (NGSP). The sensitivity of this assay was 0,05% with intra-assay coefficient of variation (CV) and inter-assay coefficient of variation (CV) precision at 2,35% and 2,66% respectively.

N^{ε}-carboxymethyllysine {(2S)-2-amino-6-(carboxymethylamino)hexanoic acid} assay. Fasting plasma samples were drown and the concentration of N^{ε}-carboxymethyllysine (CML) was measured with a novel competition-based ELISA assay using a CML-specific monoclonal antibody ELISA kit (MicroCoat, Bernried am Starnberger See, Germany). Absorbance was read using a microtitre ELISA plate reader (Sunrise™, Tecan Group Ltd, Männedorf, Switzerland) at 405 nm. CML concentrations were obtained from standard curve (linear/linear plot method) and expressed in ng/ml. All samples were run in triplicate. The sensitivity of this competitive ELISA assay was 5 ng/ml with intra-assay coefficient of variation (CV) and inter-assay CV precision at 5% and 6,8% respectively.

Statistical Analysis Statistica (version 6.0) for Windows was used for statistical analysis. The normality of value distribution was checked by Shapiro-Wilk test. Then, the results with a Gaussian distribution were analyzed with Student's t test, and those with a non-Gaussian distribution with a nonparametric Mann-Whitney U test to assess the differences between studied groups. The Spearman rank correlation test was used to evaluate the strength of association between two variables. A $p<0.05$ was considered statistically significant.

Sensitivity, specificity and the highest diagnostic value for the fructosamine, HbA_{1c}, CML level we obtain based on ROC curves analysis using MedCalc® v. 9.3.7.0 for Windows. ROC curves analyses were used to determine the optimal values of fructosamine, glycated hemoglobine (HbA_{1c}) and N^{ε}-carboxymethyllysine (CML) distinguishing between normal and disturbed glucose tolerance groups.

All results are shown as mean ± standard deviation (SD) and median (in brackets).

3. Results

In our study group, after ruling out those with any disease, patients were divided into: normoglycemic (n=30), prediabetes (n=20) and as newly diagnosed diabetes mellitus type 2 (n=8) (based on oral glucose tolerance test, see methods). The newly diagnosed diabetes type 2 were excluded than. All investigated patients were normoalbuminuria staged and eGFR > 60ml/min/m^2.

3.1. Comparison of normoglycemic (NGT) and prediabetic (PRE) subjects

Table 3 shows the baseline characteristics and clinical parameters of the elderly normal glucose tolerance subjects (NGT) and elderly prediabetes patients (PRE) according to Oral

Glucose Tolerance Test (OGTT). The groups by definition were different as far as glucose concentration was concerned (both fasting and postprandial) (p=0,0000002 and p=0,000009 respectively).

The investigated groups did not differ in clinical parameters such as arterial blood pressure and anthropometric factors (BMI, body fat, waist circumference) as well as plasma lipids.

	NGT (n=30) Mean ± SD (Median)	PRE (n=20) Mean ± SD (Median)	p
Age [years]	70,6 ± 8,4 (69,5)	70,2 ± 5,27 (70,0)	NS
BMI [kg/m^2]	27,9 ± 4,4 (26,6)	30,8 ± 5,5 (30,0)	NS
Waist [cm]	91,0 ± 7,1 (92,0)	95,5 ± 12,8 (93,0)	NS
FAT [%]	36,1 ± 17,6 (45,0)	38,3 ± 11,8 (41,5)	NS
SBP [mmHg]	136,4 ± 13,8 (135,0)	142,9 ± 11,0 (140,0)	NS
DBP[mmHg]	83,2 ± 8,8 (85,0)	78,2 ± 8,5 (80,0)	NS
G0' [mmol/l]	5,0 ± 0,5 (5,0)	5,8 ± 0,5 (5,8)	by def.: p=0,000002
G120' [mmol/l]	5,2 ± 1,2 (5,2)	7,2 ± 1,3 (7,2)	by def.: p=0,000009
Fructosamine [μmol/l]	253,5 ± 26,6 (255,0)	258,3 ± 27,8 (253,5)	NS
HbA_{1c} [%]	5,85 ± 0,40 (5,85)	6,22 ± 0,47 (6,30)	p<0,005
CML [ng/ml]	2248,7 ± 375,9 (2292,4)	2079,9 ± 418,9 (1987,0)	NS
TC [mmol/l]	4,98 ± 0,72 (5,01)	5,05 ± 0,89 (5,00)	NS
TG [mmol/l]	1,16 ± 0,50 (0,94)	1,54 ± 0,98 (1,24)	NS
HDL-C [mmol/l]	1,65 ± 0,36 (1,66)	1,46 ± 0,30 (1,44)	NS
LDL-C[mmol/l]	2,82 ± 0,71 (2,88)	2,90 ± 0,72 (2,82)	NS

p – the probability of obtaining a test statistic, SD – standard deviation, NS – not significant, by def. - by definition, NGT – normoglycemic group, PRE – prediabetic group, BMI – Body Mass Index, FAT – body fat, SBP – systolic blood pressure, DBP – diastolic blood pressure, G0' – fasting glucose, G120' - postprandial glucose, measured at 120 min after 75 grams glucose load, HbA_{1c} – glycated hemoglobin, CML – N^{ε}-carboxymethyllysine, TC – total cholesterol, TG – triglycerides, HDL-C – high density lipoproteins cholesterol, LDL-C – low density lipoproteins cholesterol,

Table 3. Baseline characteristics and clinical parameters of the normal glucose tolerance subjects (NGT) and prediabetes patients (PRE) according to Oral Glucose Tolerance Test (OGTT).

The glycated albumin - fructosamine did not differ between investigated groups (Figure 3) whereas glycated hemoglobin HbA_{1c} were higher in the prediabetes individuals (p<0,005) (Figure 4).

The CML did not differentiate normoglycemic and prediabetes elderly persons (Figure 5).

Different correlations between glycated proteins (fructosamine and HbA_{1c}) and metabolic parameters were found in analyzed subgroups (table 4 and 5 respectively) at p<0,01. There were no correlations in any investigated groups for the CML (table 6).

ROC curve analysis yielded a fructosamine concentration of the highest diagnostic value in distinguishing PRE and NGT (<=237 μmol/l, 29.0% sensitivity and 88,2% specificity,

AUC=0,511) based on OGTT (Figure 6). ROC curve analysis yielded a HbA_{1c} percentage of the highest diagnostic value in distinguishing PRE and NGT (>6,1 %, 54,8% sensitivity and 88,2% specificity, AUC=0,679) based on OGTT (Figure 7). ROC curve analysis yielded a CML concentration of the highest diagnostic value in distinguishing PRE and NGT (<=2403,2 ng/ml, 76.7% sensitivity and 41,2% specificity, AUC=0,548) based on OGTT (Figure 8).

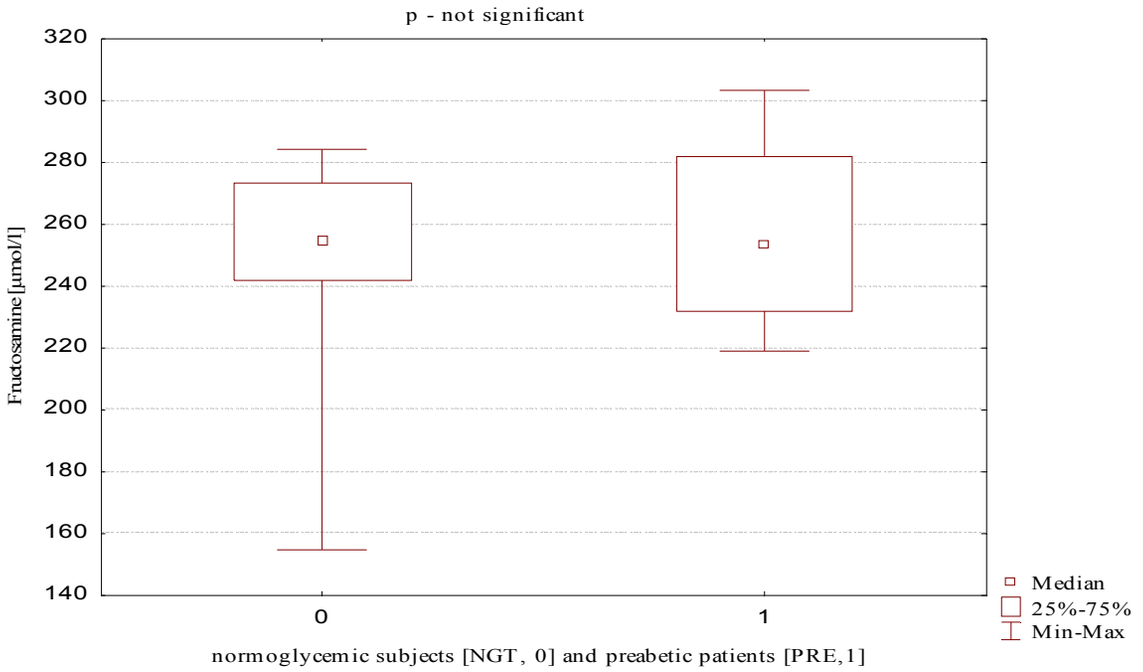

Figure 3. Comparison of fructosamine between normoglycemic subjects (NGT, 0) and prediabetic patients (PRE, 1).

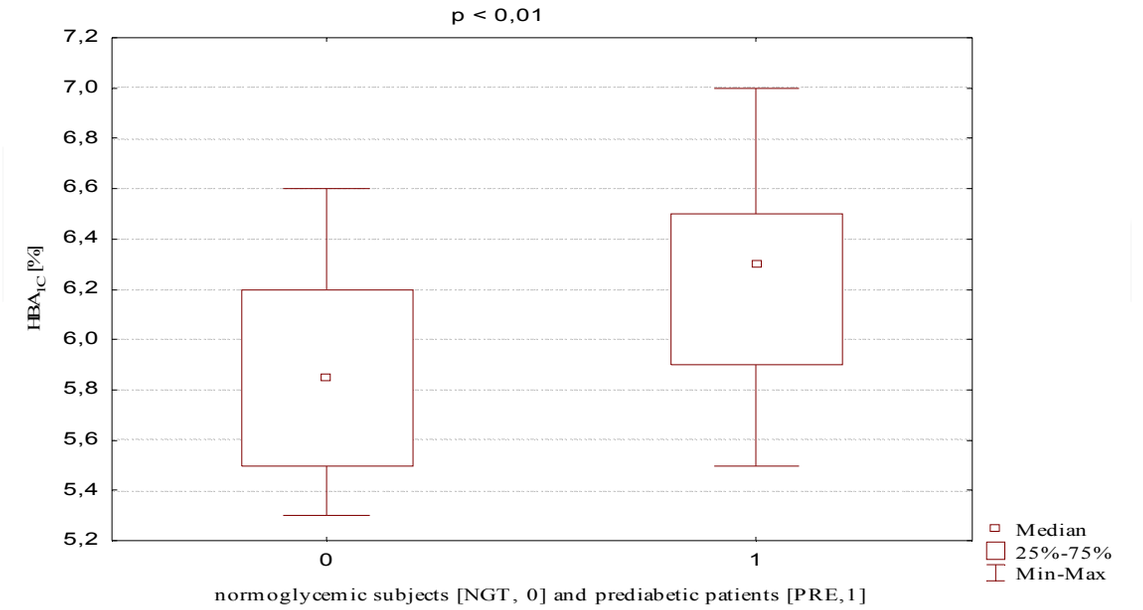

Figure 4. Comparison of glycated hemoglobin (HbA_{1c}) between normoglycemic subjects (NGT, 0) and prediabetic patients (PRE, 1).

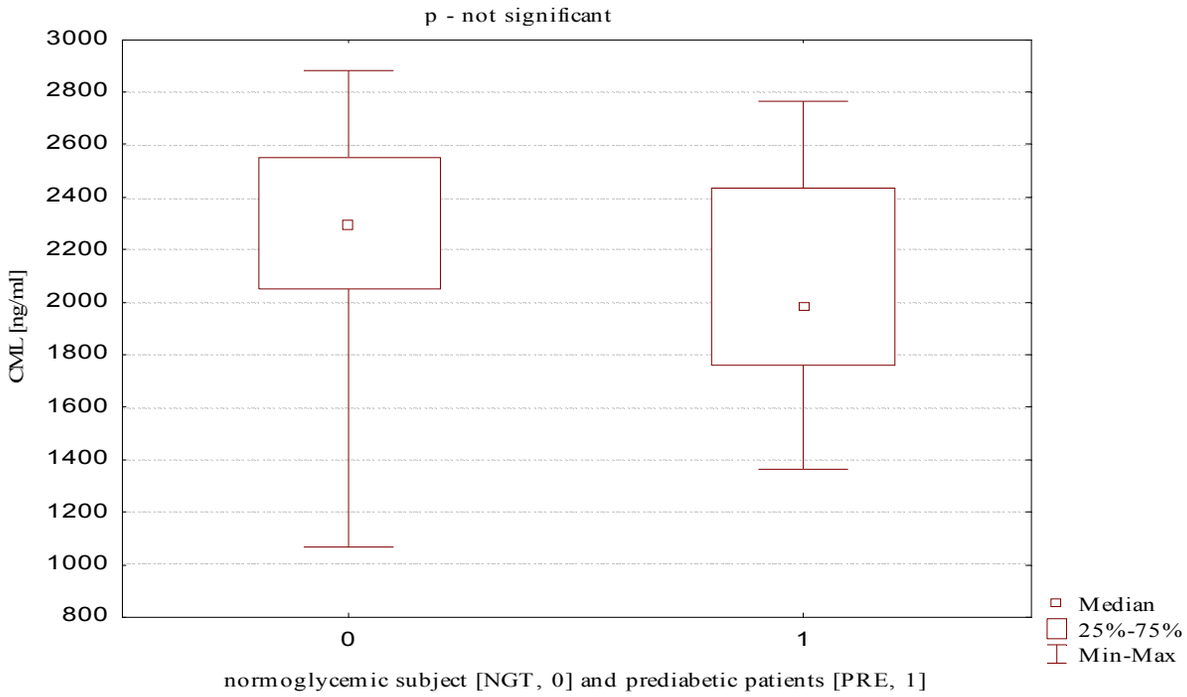

Figure 5. Comparison of CML between normoglycemic subjects (NGT, 0) and prediabetic patients (PRE, 1).

p<0,01	All persons (n=50)	NGT (n=30)	PRE (n=20)
Fructosamine & BMI	NS	NS	NS
Fructosamne & waist	NS	NS	NS
Fructosamine & FAT	R=0,39	NS	NS
Fructosamine & SBP	NS	NS	NS
Fructosamine & DBP	NS	NS	NS
Fructosamine & G0'	NS	NS	NS
Fructosamine & G120'	NS	R=0,48	NS
Fructosamine & TC	NS	NS	NS
Fructosamine & TG	NS	NS	NS
Fructosamine & HDL-C	NS	NS	NS
Fructosamine & LDL-C	NS	NS	NS

p – the probability of obtaining a test statistic, NS – not significant, NGT – normoglycemic group, PRE – prediabetic group, BMI – Body Mass Index, FAT – body fat, SBP – systolic blood pressure, DBP – diastolic blood pressure, G0' – fasting glucose, G120' (postprandial glucose) measured at 120 min after 75 grams glucose load, TC – total cholesterol, TG – triglycerides, HDL-C – high density lipoproteins cholesterol, LDL-C – low density lipoproteins cholesterol,

Table 4. Correlation for fructosamine in all investigated persons, NGT and PRE groups.

p<0,01	All (n=50)	NGT (n=30)	PRE (n=20)
HbA_{1c} BMI	NS	NS	R=-0,78
HbA_{1c} and waist	R=0,39	NS	NS
HbA_{1c} and FAT	NS	NS	NS
HbA_{1c} and SBP	NS	NS	NS
HbA_{1c} and DBP	NS	NS	NS
HbA_{1c} and G0'	R=0,45	NS	NS
HbA_{1c} and G120'	R=0,58	0,51	NS
HbA_{1c} and TC	NS	NS	NS
HbA_{1c} and TG	NS	NS	NS
HbA_{1c} and HDL-C	R=-0,47	NS	NS
HbA_{1c} and LDL-C	NS	NS	NS

p – the probability of obtaining a test statistic, NS – not significant, NGT – normoglycemic group, PRE – prediabetic group, BMI – Body Mass Index, FAT – body fat, SBP – systolic blood pressure, DBP – diastolic blood pressure, G0' – fasting glucose, G120' (postprandial glucose) measured at 120 min after 75 grams glucose load, HbA_{1c} – glycated hemoglobin, TC – total cholesterol, TG – triglycerides, HDL-C – high density lipoproteins cholesterol, LDL-C – low density lipoproteins cholesterol,

Table 5. Correlation for HbA_{1c} in all investigated persons, NGT and PRE groups.

p<0,01	All (n=50)	NGT (n=30)	PRE (n=20)
CML and BMI	NS	NS	NS
CML and waist	NS	NS	NS
CML and FAT	NS	NS	NS
CML and SBP	NS	NS	NS
CML and DBP	NS	NS	NS
CML and G0'	NS	NS	NS
CML and G120'	NS	NS	NS
CML and TC	NS	NS	NS
CML and TG	NS	NS	NS
CML and HDL-C	NS	NS	NS
CML and LDL-C	NS	NS	NS

p – the probability of obtaining a test statistic, NS – not significant, NGT – normoglycemic group, PRE – prediabetic group, BMI – Body Mass Index, FAT – body fat, SBP – systolic blood pressure, DBP – diastolic blood pressure, G0' – fasting glucose, G120' (postprandial glucose) measured at 120 min after 75 grams glucose load, CML – N^{ε}-carboxymethyllysine, TC – total cholesterol, TG – triglycerides, HDL-C – high density lipoproteins cholesterol, LDL-C – low density lipoproteins cholesterol,

Table 6. Correlation for CML in all investigated persons, NGT and PRE groups.

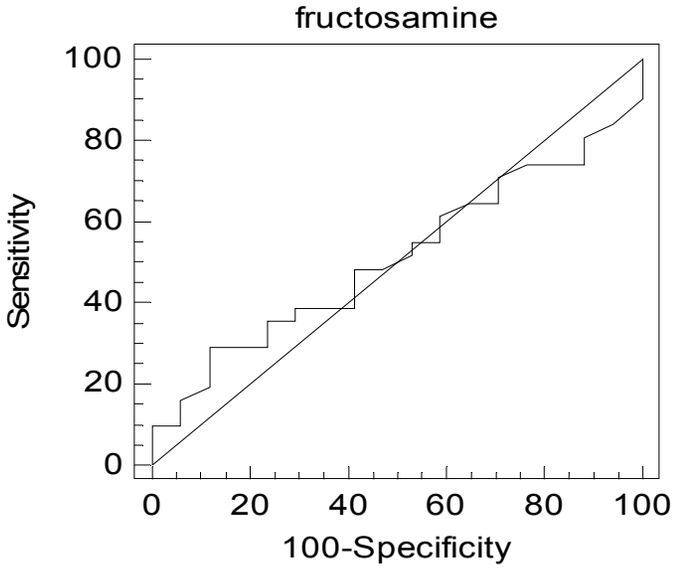

Figure 6. Receiver operating characteristic (ROC) curve for fructosamine diagnostic value (sensitivity and specificity) in distinguishing between normal glucose tolerance subjects (NGT) and prediabetic patients (PRE) classified according to OGGT results.

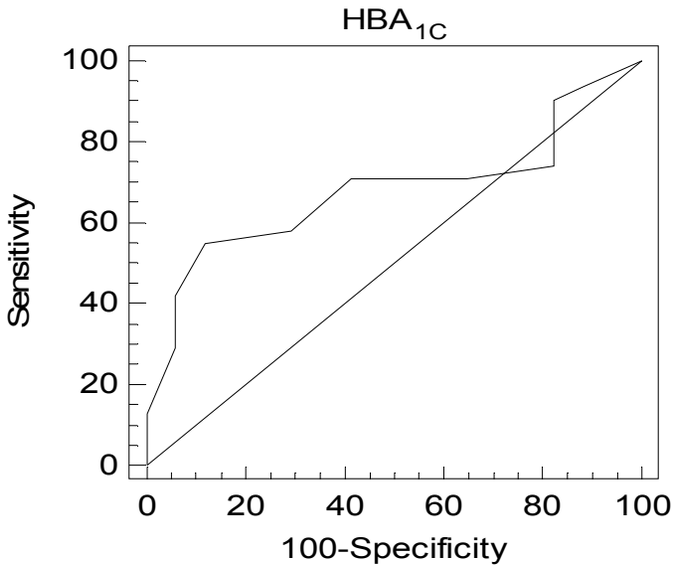

Figure 7. Receiver operating characteristic (ROC) curve for HbA_{1c} diagnostic value (sensitivity and specificity) in distinguishing between normal glucose tolerance subjects (NGT) and prediabetic patients (PRE) classified according to OGGT results.

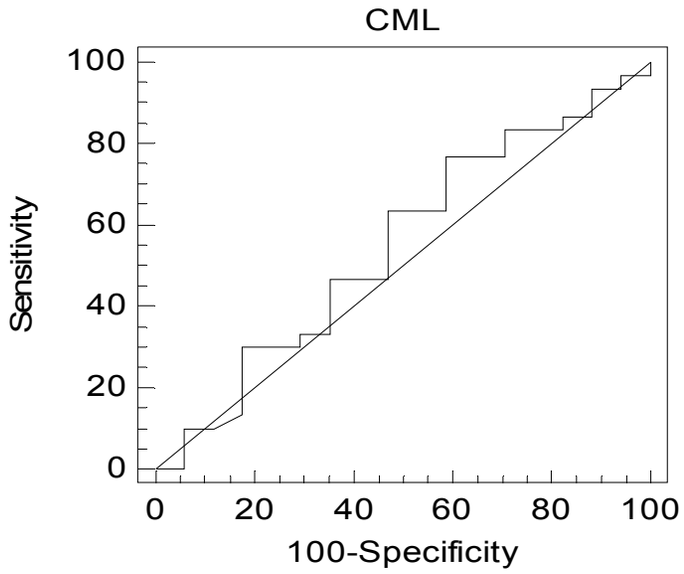

Figure 8. Receiver operating characteristic (ROC) curve for CML diagnostic value (sensitivity and specificity) in distinguishing between normal glucose tolerance subjects (NGT) and prediabetic patients (PRE) classified according to OGGT results.

3.2. Comparison of two prediabetic (PRE) subgroups: Impaired Fasting Glucose (IFG) and Impaired Glucose Tolerance (IGT) subjects

Table 7 shows the baseline characteristics and clinical parameters of the Impaired Fasting Glucose subjects (IFG) and Impaired Glucose Tolerance subjects (IGT) subgroups of prediabetic elderly patients according to Oral Glucose Tolerance Test (OGTT). The subgroups by definition were different as far as glucose concentration was concerned (both fasting and postprandial) (p=0,01 and p=0,001 respectively). Otherwise the prediabetic subgroups did not differ in clinical parameters as well as biochemical factors. However the glycated hemoglobin (HbA_{1c}) had tendency to be higher in the Impaired Glucose Tolerance individuals and the glycated albumin - fructosamine and Advanced Glycation End Product - N^{ε}-carboxymethyllysine had tendency to be higher in Impaired Fasting Glucose subjects.

4. Disscusion

One of the fundamental elements for establishing prevention, treatment and prognosis of diabetes mellitus is an assessment of the clinical stage of glucose metabolism problems.

Dysglycemia population is recognized with increased cardiovascular morbidity and mortality [Khaw KT. Et al., 2004; Sung J. et al. 2009). The cumulative effect of many metabolic risk factors (clinical and biochemical) in one individual which influenced cardiovascular risk are widely discussed (Selvin E. et al., 2005; Gerstein HC. et al., 2005). In our study we investigated elderly otherwise healthy persons. We found that in those with the increased risk for the type 2 diabetes mellitus (prediabetes) there were more (but not significant) indicators for the metabolic syndrome (obesity, abdominal obesity, systolic hypertension).

	IFG (n=8) Mean ± SD (Median)	IGT (n=12) Mean ± SD (Median)	p
Age [years]	69,8 ± 3,8 (69,0)	70,2 ± 6,56 (70,0)	NS
BMI [kg/m^2]	30,4 ± 6,8 (29,0)	31,7 ± 4,6 (30,5)	NS
Waist [cm]	96,0 ± 13,8 (91,5)	96,5 ± 12,9 (94,0)	NS
FAT [%]	35,3 ± 12,6 (38,8)	39,8 ± 12,2 (44,4)	NS
SBP [mmHg]	141,7 ± 12,1 (142,5)	142,0 ± 10,8 (140,0)	NS
DBP[mmHg]	76,7 ± 8,8 (72,5)	79,0 ± 9,1 (80,0)	NS
G0' [mmol/l]	6,3 ± 0,5 (6,4)	6,3 ± 0,5 (6,8)	by def.: p=0,01
G120' [mmol/l]	6,6 ± 1,0 (7,0)	8,8 ± 1,0 (9,1)	by def.: p=0,001
Fructosamine [μmol/l]	261,8 ± 27,5 (267,0)	257,0 ± 30,5 (253,5)	NS
HbA_{1c} [%]	6,04 ± 0,60 (5,90)	6,35 ± 0,37 (6,40)	NS
CML [ng/ml]	2123,9 ± 375,5 (2122,7)	2053,4 ± 443,1 (1987,0)	NS
TC [mmol/l]	4,85 ± 0,86 (4,74)	5,41 ± 0,89 (5,25)	NS
TG [mmol/l]	1,22 ± 0,47 (1,18)	1,73 ± 1,2 (1,14)	NS
HDL-C [mmol/l]	1,53 ± 0,42 (1,50)	1,51 ± 0,32 (1,50)	NS
LDL-C[mmol/l]	2,76 ± 0,83 (2,92)	3,09 ± 0,61 (2,87)	NS

p – the probability of obtaining a test statistic, SD – standard deviation, NS – not significant, by def. - by definition, NGT – normoglycemic group, PRE – prediabetic group, BMI – Body Mass Index, FAT – body fat, SBP – systolic blood pressure, DBP – diastolic blood pressure, G0' – fasting glucose, G120' - postprandial glucose, measured at 120 min after 75 grams glucose load, HbA_{1c} – glycated hemoglobin, CML – N^{ε}-carboxymethyllysine, TC – total cholesterol, TG – triglycerides, HDL-C – high density lipoproteins cholesterol, LDL-C – low density lipoproteins cholesterol,

Table 7. Baseline characteristics and clinical parameters of the prediabetes (PRE) subgroups the Impaired Fasting Glucose subjects (IFG) and the Impaired Glucose Tolerance subjects (IGT) according to Oral Glucose Tolerance Test (OGTT).

We had hoped while performing those analysis to find a set of laboratory measures that would provide a better indicator of a prediabetic elderly persons for glycemic control than any one measure alone.

The amount of glycated proteins (fructosamine and HbA_{1c}) depend on time-averaged glucose concentration. Thus fructosamine and HbA_{1c} reflect the extent of exposure to glucose in the 2-4 and 8-12 weeks before testing, respectively. Since 2010, American Diabetes Association has added a new recommendation for diagnosis of diabetes mellitus, concerning not only based on hyperglycemia (random, fasting or due to OGTT) but also glycated hemoglobin HbA_{1c} (ADA 2010).

The primary aim of the study was to evaluate the blood levels of glycated proteins and CML in two clinical situations, normal glucose tolerance and prediabetic state in elderly patients. Our study showed that the glycated hemoglobin corresponded better in distinguishing the NGT from PRE in the investigated elderly persons than fructosamine or advanced glycation end product – N^{ε}-carboxymeethyllysine.

Hyperglycemia and oxidative stress accelerate not only glycation of proteins such as hemoglobin but also leads to Advanced Glycated End Products (AGEs) such as N^{ε}-carboxymethyllysine (CML). During natural aging AGEs accumulate and the concentration of formers depends on time and progressive reduction in the capacity to neutralise oxidative stress and may leads to many complications such as atherosclerosis and dementia (Hipkiss AR., 2006; Semba RD. et al., 2010). In 2003 Hamelin M. and collegues established that CML are increased in the rat serum with aging (Hamelin M. et al., 2003). So is CML independent risk factor for aging or rather diseases? Our study focused on CML in elderly group at the earliest stages of hyperglycemia. Our investigated groups had no known clinical and laboratory complications, in all subgroups CML concentration is very high in comparison with others investigators. Ahmed K.A. et al. excluded the effect of age on CML concentration in type 2 diabetic patients with ischemic heart disease (Ahmed K.A. et al., 2007). They argued that increased CML concentration was an independent from other risk factors for the coronary artery disease (CAD) in type 2 diabetes mellitus and ha significant predictive power to CAD especially in type 2 diabetes mellitus (Ahmed K.A. et al., 2008). In our investigated groups CML concentration were higher in comparison with CML concentration in Ahmed work. Our investigated study patients were either with normal glucose metabolism or prediabetes stage, both elderly patients with no clinical complications such as CAD or kidney failure. CML concentration depends on proteins glycation but also is directly proportional to creatinine concentration and inversely proportional to glomerular filtration rate. Hirata K. work showed that CML is increased in type 2 diabetes mellitus patients with kidney failure in comparison with type 2 diabetes mellitus patients with normo-, micro- or even macroalbumnuria (Hirata K. & Kubo K. 2004). While Wagner Z. et collegues demonstrated normal levels of CML and AGEs (fluorescence) in patients with type 2 diabetes mellitus who had normal renal function (Wagner Z. et al., 2001). Since we excluded patients with CAD or kidney disturbances, those factors did not influences CML concentration, in plasma in our investigated groups. Thus, the determined CML concentration in plasma did not follow increased accumulation in the body. Baumann M. and colleagues found that CML might be accumulated in tissues, what could lead to different metabolic complications (Baumann M., 2009).

Interesting work showed Dworacka M. et colleagues. Their results revealed significantly higher CML in non-diabetic patients with coronary heart disease than in healthy control subjects and were comparable to serum CML in patients with type 2 diabetes mellitus without late complications and coronary heart disease (Dworacka M. et al., 2002). In our study we realized higher (but not significant) CML concentration in subgroup defined as normoglycemic. This patients had lower total cholesterol, LDL-C and triglicerydes and higher HDL-C in comparison with prediabetics. Thus it is possible that complications of diabetes mellitus are themselves related to pathobiochemical alterations other than protein glycation i.e. oxidative stress and lipids peroxidation.

There is no doubt that elevated CML are closely associated with late complication of hyperglycemia in patients with type 2 diabetes mellitus or atherosclerotic angiopathies.

Further studies should be performed in patients with the very early stages of dysglycemia to find when the pathology starts.

Different tendencies for HbA_{1c} and fructosamine and CML were observed while IFG or IGT were separated (as in table 7). Based on HbA_{1c} IGT group seems to be more advanced on the way to diabetes. However IFG individuals tended to present higher fructosamine and CML which may reflect not only glycation. There is no literature data concerning different metabolic aspect of IFG or IGT in elderly, but in middle aged population Faerch K. et al. showed different insulin action and incretin hormone concentration suggesting different management according to the prediabitic subgroup diagnosis (Faerch K. et al., 2008).

Despite some answers our study has brought, that there are some challenges for the future investigations that should be coped. What biomarker could predict the increased risk related to dysglycemia in elderly patients? Does the kind of prediabetes state matter, in a context of metabolic complications, especially in elderly patients?

5. Conclusions

There is no doubt that elevated fructosamine, HbA_{1c} and CML are closely associated with late complication of hyperglycemia in patients with type 2 diabetes mellitus or atherosclerotic angiopathy. The best marker in dysglycemic but not yet diabetic elderly patients seems to be only glycated hemoglobin. When N^{ε}-carboxymethyllysine and fructosamine as a single marker seem to be not good enough prognostic indicators of dysglycemia. In apparently healthy elderly people otherwise with no diagnosed comorbidities but with hyperglycemia, clinical assessment and laboratory investigations of hyperglycemia complications may help in prevention all global complications. In our opinion CML and/or fructosamine may result from different metabolic pathways (i.e. glycation and oxidative stress).

Author details

Sylwia Dzięgielewska-Gęsiak and Ewa Wysocka
Department of Clinical Biochemistry and Laboratory Medicine, Chair of General Chemistry and Clinical Biochemistry, Poznan University of Medical Sciences, Poznan, Poland

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