Diabetes, obesity, metabolic syndrome

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COULD I-FABP BE AN EARLY MARKER OF CELIAC DISEASE IN CHILDREN WITH TYPE 1 DIABETES MELLITUS?

A. Ochocińska [1], E. Konopka [3], J. Bierła [3], A. Groszek [2], M. Wysocka-Mincewicz [2], B. Cukrowska [3]

[1]*Department of Biochemistry, Radioimmunology and Experimental Medicine, The Children's Memorial Health Institute, Warsaw*

[2]*Department of Endocrinology and Diabetology, The Children's Memorial Health Institute, Warsaw*

[3]*Department of Pathomorphology, The Children's Memorial Health Institute, Warsaw*

BACKGROUND-AIM

Objective: Patient with diabetes mellitus type 1 (T1DM) are at risk of celiac disease (CD). Epithelial barrier seems to play an important role in activation of processes leading to autoimmunity including celiac disease (CD) and type 1 diabetes mellitus (T1DM).
The aim of this study was to analyze the level of intestinal fatty acid binding protein (I-FABP) – a serological biomarker of an impaired epithelial barrier in pediatric patients with T1DM.

METHODS

Methods: Study included patients with T1DM (n=156), with T1DM and active CD (T1DM-CD, n=42), with active CD only (n=25), and age-matched healthy children (n=55). In the T1DM-CD group, patients with negative CD serology one year before CD diagnosis (T1DM-CD-1, n=20), were distinguished. I-FABP measurement was also performed in CD and T1DM-CD patients after at least 6 months of gluten free diet (GFD). The sera were tested using immunoenzymatic assay (pg/mL, Hycult Biotech Inc., USA).

RESULTS

Results: The significantly increased levels of I-FABP were found in T1DM, active CD and T1DM-CD groups (1153±665, 1178±1002, 1093±720, respectively) compared with controls (485±416, $p<0,05$). GFD induced a significant decrease in I-FABP levels to control values in CD and T1DM-CD groups (346±266 and 543±448, respectively). Interestingly, in T1DM-CD-1 and T1DM, I-FABP levels were comparable (846±382 and 1153±665, respectively), and significantly increased to control and T1DM-CD values on GFD.

CONCLUSIONS

Conclusions: The results indicate that epithelial barrier is disrupted in T1DM patients independent on CD development so I-FABP cannot be an early marker of CD in T1DM patients. Although GFD can improve epithelial it is still open question whether GFD could have beneficial effect on intestinal barrier in early stages of T1DM.
The study was financed by IPCZD grants: S156/2017, M32/2018, M41/19.

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EVALUATION OF SEKISUI RC-W HPLC ANALYZER FOR THE MEASUREMENT OF HBA1C

E. Urrechaga [1], P. Pellicer [1], M. Merino [1], G. Mugertza [1], C. Ponga [1]
[1]*Hospital Galdakao Usansolo*

BACKGROUND-AIM

Sekisui RC-W is a compact High pressure liquid chromatography system for HbA1c tests designed for point-of-care settings. The method is reverse-phase cationic exchange chromatography with continuous gradient of the moving phase. The analysis time is 5 minutes per sample. The volume required is 5 µL, both capillary or venous blood can be used.
The analytical performance was evaluated to verify quality of results.

METHODS

Carry over, reproducibility and method comparison were evaluated according to CLSI EP-10, EP-5 and EP-9 guidelines.
Within run imprecision was studied running samples 10 times.
Effect of the total Hb concentration was studied by sequential dilutions and coexistent interfering substances was studied adding increasing concentrations of Glucose, Sodium Cyanide or Acetyl Aldehyde solutions to pooled samples, incubated at 37ºC two hours and analyzed.
Correlation with ADAMS A1c HA-8180V; 105 samples were run in parallel and Passing - Bablok regression applied to the couples of results. Samples with Hb variants were also analysed.

RESULTS

Carry over 0.01 % (113 y 29 mmol/mol).
Imprecision within run CV (samples) mean 29 mmol/mol 0.48 %; mean 113 mmol/mol 0.53 %.
Reproducibility Within run CV 33 mmol/mol 0.01 %; 91 mmol/moll 0.02 %.
Between run CV 33 mmol/mol 0.57 %; 91 mmol/mol CV 0.29 %.
Between day CV 33 mmol/mol 0.93 %; 91 mmol/mol 0.48 %.
Total CV 33 mmol/mol total CV 1.23 %; 91 mmol/mol total CV 1.8 %.
HbA1c concentration 37 mmol/mol was not affected by Hb in the range 200- 55 g/L.
HbA1c concentration 31 mmol/mol was not affected by labile A1c fraction 195 mmol/mol carbamylated Hb 86 mmol/mol nor acetylated Hb 58 mmol/mol.
Method comparison range 25-135 mmol/mol R=0.999
Y=1.063x -0.32 95% Confidence Interval slope 1.049-1.074 intercept -0.39-0.24
Mean difference 0.92 mmol/mol. Hb variants were detected, S, C, D and E peaks were correctly identified.

CONCLUSIONS

The overall analytical quality of results is according to the documents of consensus.
The RC-W analyzer simplifies diabetes managements in clinics, doctor's offices, wards and small labs, delivering high quality and clinically relevant results to help physicians improve patient decision-making and diabetic patient compliance.

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EFFECT OF HEMOLYSIS ON HBA1C MEASUREMENT BY THE ATELLICA DCA ANALYZER

K. Das [1], C. Tilghman [1]
[1]*Siemens Healthcare Diagnostic Inc, 2 Edgewater Drive, Norwood, MA, USA*

BACKGROUND-AIM

An estimated 3.3% of all venous sample rejection reported is due to pre-analytical hemolysis. Up to 43% of fingerstick samples were reported to have pre-analytical hemolysis. The hemolysis effect varies among assays. (Lippi et. al. Clin Chem Lab Med. 2008;46:764-72). Atellica® DCA HbA1c assay, hemolyzes samples in the first step of the measurement process and sample hemolysis has little effect on Atellica DCA HbA1c assay. In this poster, we report evaluation of the pre-analytical hemolysis effect on Atellica DCA HbA1c assay.

METHODS

Four blood samples with various HbA1c levels, 5.3%, 6.6%, 7.1% and 7.6%, were tested. For each HbA1c level, a set of five test samples was prepared with various hemolysis levels by mixing hemolyzed and non-hemolyzed blood in different ratios. Respective non-hemolyzed sample was used as a control. Hemolysis indices were estimated using a CDC approved color chart. The HbA1c of the control and test samples was measured in 30 replicates using Atellica DCA analyzers. Analysis included (1) regression analysis for HbA1c vs. hemolysis indices, (2) paired-bias analysis between the control and each hemolysis level and (3) comparing mean HbA1c of each hemolysis with standard-deviation range of the respective control.

RESULTS

Slopes for regression analyses for the four samples were -0.00099, 0.0018, 0.0036 and 0.0016 respectively. The slopes were statistically not different from zero (p value > 0.05) for clinically relevant hemolysis levels. The bias values were -0.95% for the completely hemolyzed first sample and 1.51%, 0.53%, 0% for the second, third and fourth samples respectively at 500-1000 mg/dL hemolysis level. The mean HbA1c of 30 replicates for all four samples at all hemolysis levels fell within the ±2SD range of the respective control sample.

CONCLUSIONS

The median hemolysis index is reported as 210 mg/dL. The results indicate that the hemolysis effect on Atellica DCA HbA1c assay, is minimal and outside the clinically significant levels.

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IMPACT OF ZINC TO COPPER RATIO AND LIPOCALIN 2 IN OBESE PATIENTS UNDERGOING SLEEVE GASTRECTOMY

H. Demerdash [1], A. Sabry [2], O. Arida [2]
[1]*Clinical Pathology Department Alexandria University Hospitals*
[2]*Surgery Department Alexandria University Faculty of Medicine*

BACKGROUND-AIM

Obesity constitutes an enormous health struggle worldwide. There is conspicuous recognition of influence of subclinical inflammation on nutritional status in obesity. Lipocalin 2 (Lcn2) is an adipokine abundantly expressed in adipose tissue predominantly in response to metabolic stress. It has a potential role in metabolic dysfunction. In this study we investigated the value of zinc (Zn) to copper (Cu) ratio, serum Lcn2 as promising biomarkers of nutritional imbalance in subjects with morbid obesity undergoing laparoscopic sleeve gastrectomy (LSG).

METHODS

A prospective cohort study was conducted, recruiting 107 subjects with morbid obesity (69 women, 38 men) and age ranging from 20 to 55 years old. Anthropometric measurements and Laboratory investigations were performed preoperatively and nine months postoperatively. Blood samples were collected for determination of Lcn2 and insulin were determined by ELISA. Zn and Cu levels were evaluated by atomic absorption/emission spectrometeras, while Serum CRP, Serum iron, and ferritin were measured using a Hitachi autoanalyzer 704.

RESULTS

Preoperative Zn deficiency was 16.82% increased to 22.43% postoperatively. None of studied subjects exhibited Cu deficiency in the two consecutive measurements. While prevalence of preoperative iron deficiency was10.28% increased to 15.89% postoperatively. Lipids, HOMA-IR, inflammatory sensitive proteins as Lcn2, CRP and ferritin were reduced at end of study period. There was negative correlation between delta change in body weight and Lcn2, leptin and HOMA-IR. On the other hand, it was positively correlated with Zn/Cu ratio.

CONCLUSIONS

owing to existence of subclinical inflammation, TE concentrations should be elucidated with caution to avoid misdiagnosis of deficiency. Also pursuing systemic inflammation simultaneously with vitamin supplementation in pre and post bariatric surgery management. Lcn2 and Zn/Cu ratio may be utilized as potential biomarker of nutritional status and metabolic improvement after LSG.

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THE ASSOCIATION BETWEEN ATHEROGENIC INDEX AND OBESITY IN OLDER PATIENTS

G.I. Constantin [1], C.M. Pena [1], S. Opris [1]
[1]*"Ana Aslan" National Institute of Gerontology and Geriatrics*

BACKGROUND-AIM

Obesity is the most common metabolic disease, characterized by an inflammatory condition with excess adipose tissue and low levels of adiponectin and is often associated with severe chronic diseases such as cardiovascular disease, hypertension and diabetes. Relevant abnormalities were found in lipid metabolism by changing the values of cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides in obese patients. A good indicator used in obesity, to evaluate the lipid profile and commonly used as optimal indicator of dyslipidemia and associated diseases, is the atherogenic index.

METHODS

Our study aimed to determine the levels of lipid profile and assess atherogenic index in two study groups of older patients (aged 67 ± 8 years): a group of patients with obesity and a group of control patients. Serum determinations of biochemical parameters (blood glucose, total cholesterol (TC), high density lipoproteins (HDL-C), low density lipoproteins (LDL-C), triglycerides (Tg)) were performed by laboratory tests using standardized methods. Atherogenic index was calculated by using the following formula: log (TG/HDL-C).

RESULTS

In our study was observed a significantly high levels in atherogenic index in patients with obesity compared to control group (0.629±0.25 vs 0.320±0.22 mg/dL serum).

CONCLUSIONS

Our results suggest that the atherogenic index could be considered as a novel and convenient clinical biomarker for the detection of obesity, in the incidence and prognosis of associated diseases, and can be considered a good predictor for monitoring and follow-up in the treatment of dyslipidemia in obese patients.

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COMPARATIVE ANALYSIS OF THE GLUCOSE DETERMINATION BY THREE METHODS

M. Zárate [1], M. Giménez Blanco [1], R. Rubio Sánchez [1], M.d.M. Viloria Peñas [1]
[1]*Hospital Universitario Virgen de Valme*

BACKGROUND-AIM

Glucose is the most performed biochemical determination in the laboratory of our hospital. In recent years, Point-of-Care (POC) equipment are emerging, allowing the determination of patients' blood glucose at the time and place of health care, by non-laboratory personnel. In our laboratory, blood glucose is routinely determined in the Cobas c 702 analyzer. Besides, we have two different POC glucometers (Accu-Chek Inform II and Accu-Chek Performa) that allow measuring this determination quicker.

METHODS

Samples from 70 patients were analyzed: peripheral blood in the 2 POC devices (with test strips) and serum in Cobas c 702. The serum was obtained after centrifugation of the patients' blood collected in tubes with inert separator gel and coagulation activator (SST II Advance tubes).

RESULTS

Cobas-Inform: 1) Passing-Bablock regression (CI95%): intercept = 4.0000 (-7.7931–15.2712) and slope = 1.0000 (0.8814–1.1207). 2) Bland-Altman plot (+/- 1.96 SD): mean difference = 0.97 (0.88–1.07). 3) Pearson correlation coefficient: R = 0.9697.
Cobas-Performa: 1) Passing-Bablock regression (CI95%): intercept = 10.4545 (2.0000-20.6000) and slope = 0.9091 (0.80000-1.0000). 2) Bland-Altman plot (+/- 1.96 SD): mean difference = 0.99 (0.90-1.10). 3) Pearson correlation coefficient: R = 0.9714.
Inform-Performa: 1) Passing-Bablock regression (CI95%): intercept = 4.9565 (-1.0000-12.6667) and slope = 0,9348 (0.8667-1.0000). 2) Bland-Altman plot (+/- 1.96 SD): mean difference = 1.02 (0.94-1.11). 3) Pearson correlation coefficient: R = 0.9765.

CONCLUSIONS

The results obtained in the Cobas c 702 analyzer and the Accu-Chek Inform II glucometer are interchangeable since there are no systematic or proportional differences. In contrast, the results obtained in the Cobas c 702 analyzer and the Accu-Chek Performa glucometer are not interchangeable, since there are systematic, although not proportional, differences. The use of POC equipment outside the laboratory allows health professionals to obtain results at the time and place of patient care, and it is not necessary to send samples to the laboratory, accelerating diagnosis and medical treatment.

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EFFECT OF LABILE GLYCATED HEMOGLOBIN ON HBA1C MEASUREMENT BY THE ATELLICA® DCA ANALYZER

K. Das [1], C. Tilghman [1], J. Mayfield [2]
[1]*Siemens Healthcare Diagnostic Inc, 2 Edgewater Drive, Norwood, MA, USA*
[2]*Siemens Healthcare Diagnostic Inc, 430 S. Beiger Street, Mishawaka, IN, USA*

BACKGROUND-AIM

Research indicated labile glycated hemoglobin (L-A1c) concentration was increased after meal and fluctuated with increasing blood glucose level. Such increase in L-A1c is also reported to cause false HbA1c results on HPLC and boronate affinity methods. The immunoturbidimetry based HbA1c assays measure HbA1c-protein itself by specific immunobinding. Therefore, in theory, an increase in labile-glycated hemoglobin is not expected to influence an immunoturbidimetry assay. In this poster we report the effect of the increased L-A1c concentration on HbA1c results reported on immunoturbidimetry based Atellica® DCA and boronate affinity based AFINION 2 analyzer.

METHODS

Whole blood samples with fixed HbA1c levels ranging from 4.7% to 8.1%) were tested at various elevated L-A1c concentrations (1-4%, 10-14%, >14%). The elevated L-A1c concentrations were achieved by glycating whole blood in various in-vitro conditions. The HbA1c values of the control and the glycated samples were measured in five replicates on TOSOH G8, AFINION 2, and Atellica DCA analyzers. The average HbA1c on TOSOH G8 analyzer of the non-glycated sample was considered as the reference HbA1c for the control sample. The HbA1c percent bias value was calculated for each device at the elevated L-A1c concentrations compared to the reference value of the control sample.

RESULTS

The HbA1c percent bias for samples tested on the Atellica DCA analyzer were in the range from -5.38% to +4.23% when compared to the reference HbA1c value of the respective control samples for all the samples tested. The percent bias for the samples tested on AFINION 2 analyzer were -28.85% to 2.85%.

CONCLUSIONS

The results of this study indicated that Atellica DCA analyzer reported HbA1c results within acceptable bias ranges with increasing L-A1c concentration of a sample. Conversely, AFINION 2 analyzer reported high HbA1c results which were outside the acceptable range with the increasing L-A1c concentrations. The results indicate Atellica DCA analyzer measurements can be made regardless of meal timing or blood sugar level, while boronate assays may have a negative impact under the same condition due to the presence of elevated level of L-A1c.

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REFERENCE INTERVALS OF GLYCATED ALBUMIN IN PREGNANT WOMEN: AN ITALIAN STUDY

L. Agnello [1], G. Bivona [1], B. Lo Sasso [1], C. Scazzone [1], M. Pantuso [1], E. Fatta [3], A.M. Ciaccio [3], M. Vidali [2], M. Ciaccio [1]
[1]*Department of Biomedicine, Neurosciences and Advanced Diagnostics, Institute of Clinical Biochemistry, Clinical Molecular Medicine and Laboratory Medicine, University of Palermo, Palermo, Italy.*
[2]*Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.*
[3]*University of Palermo, Palermo, Italy.*

BACKGROUND-AIM

Glycated albumin (GA) has great potential as a biomarker of glycaemic status in pregnancy. The reference intervals (RIs) of GA have been well established in Oriental healthy pregnant women, but few data are available on the Caucasian population. The aim of this study was to establish the GA RIs in Italian pregnant women, without overt diabetes mellitus (DM) or gestational diabetes mellitus (GDM), and to compare it with RIs of healthy women not in pregnancy.

METHODS

Methods. This study was performed at the University Hospital “P. Giaccone” of Palermo, Italy. Eligible patients were all consecutive pregnant women (age ≥18 years) without GDM or overt DM attending the Unit of Gynaecology and Obstetrics for routine prenatal clinical care. We also included healthy blood donors’ women not in pregnancy with basal glycaemia ≤5.5mmol/L.

RESULTS

A total of 170 healthy pregnant women (median age 29 years, first trimester n=32, second trimester n=73, third trimester n=65) and 363 healthy women (median age 44 years) were included. GA levels were significantly higher in pregnant than in healthy women (12.7% vs 12.2%; p<0.001) and significantly different among the trimesters of pregnancy (trim1:12.9%, trim2:13.0%, trim3:12.2%; overall p=0.001). In particular, taking into account Bonferroni’s correction, healthy women displayed GA levels comparable to pregnant women in the third trimester and significantly lower than pregnant women in the first two trimesters (respectively p=0.036 and p=0.001). Moreover, pregnant women in the third trimester displayed significantly lower levels than women in the second trimester (p=0.001). No other difference among trimesters was significant. Lower and upper reference limits of the RIs in healthy women were 10.00 (90%CI 9.80-10.20) and 14.54 (90%CI 14.30-14.90), while among trimester subgroups, were, respectively, 10.04 (90%CI 9.22-10.92) and 15.91 (90%CI 15.26-16.61) in the first trimester, 10.43 (90%CI 10.00-10.91) and 15.46 (90%CI 15.01-15.91) in the second trimester, 9.85 (90%CI 9.50-10.25) and 14.49 (90%CI 14.05-14.93) in the third trimester.

CONCLUSIONS

In this study, we established RIs of GA in Caucasian healthy pregnant women. The next step is to assess the clinical usefulness of GA in pregnant Caucasian women.

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SERUM PRO-OXIDANT-ANTIOXIDANT BALANCE LEVELS ARE INDEPENDENTLY AND INVERSELY ASSOCIATED WITH TYPE 2 DIABETES IN ADULT POPULATION

A. Klisic [2], A. Ninic [1], J. Kotur-Stevuljevic [1]
[1]*Department for Medical Biochemistry, University of Belgrade - Faculty of Pharmacy, Belgrade, Serbia*
[2]*Primary Health Care Center, University of Montenegro-Faculty of Medicine, Podgorica, Montenegro*

BACKGROUND-AIM

Prediabetes and type 2 diabetes mellitus (T2DM) with good glycemic control are not explored thoroughly in relationship with oxidative stress (OS). The aim of this study was to explore the association of several OS byproducts [i.e., advanced oxidation protein products (AOPP), total oxidant status (TOS), total antioxidant status (TAS), total protein sulfhydryl groups (tSGH) and prooxidant-antioxidant balance (PAB)] with prediabetes and T2DM with good glycemic control in adult population.

METHODS

A total of 60 patients with prediabetes (21 men and 39 women) and 91 T2DM patients (51 men and 40 women) were included. Relationship between clinical data and glycated haemoglobin (HbA1c) was examined using Spearman's correlation analysis. Associations between examined markers and diabetes presence were tested by univariate and multivariate binary logistic regression analysis.

RESULTS

TAS levels were higher (P=0.002) and PAB levels were lower (P=0.003) in T2DM patients compared to group with prediabetes. TOS, AOPP and tSHG did not differ between groups. HbA1c levels correlated positively with TAS (ρ=0.186, P=0.031). Negative correlation was determined between HbA1c and PAB levels (ρ= -0.325, P=0.004). PAB levels were associated with diabetes presence in univariate analysis [OR=0.987, 95% CI=0.978-0.995, P=0.003]. When tested in model with covariates age, gender and body mass index, PAB levels kept their independent negative association with diabetes [OR=0.988, 95% CI=0.978-0.998, P=0.021].

CONCLUSIONS

PAB levels, as a marker of increased pro-oxidant burden were lower and TAS, as a marker of increased antioxidative defence were higher in T2DM with good glycemic control compared to patients with prediabetes.

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IMPLEMENTATION OF VACUETTE®FCMIX BLOOD COLLECTION TUBES FOR THE CORRECT SCREENING AND DIAGNOSIS OF GESTATIONAL DIABETES.

J. Rodríguez [1], C. Fernández-Prendes [1], M.A. Huertas [1], E. Fuentes [1], M. Doladé [1], M. Martínez-Bujidos [1]
[1]*Hospital Germans Trias i Pujol, Spain*

BACKGROUND-AIM

The breakdown of glucose (glycolysis) in blood samples plays an important role in glucose measurement, particularly in the diagnosis of diabetes mellitus and gestational diabetes mellitus (GDM). Tubes with citric-citrate/FX/EDTA additives stabilize in-vivo glucose levels in whole blood immediately after collection, being recommended by the American Diabetes Association (ADA).
The aim of this study was to compare tubes with citric-citrate/FX/EDTA (VACUETTE®FCMix, Greiner bio-one (FCMix)) and tubes without additives (WAD), in order to implement FCMix tubes for the diagnosis of GDM.

METHODS

Blood was obtained from 41 pregnant patients under oral glucose tolerance test. From each patient four FCMix and four WAD tubes were obtained at basal, 60 min, 120 min and 180 min. All samples were centrifuged at 3000rpm for 10 min and glucose was measured by spectrophotometry (AU5800, Beckman Coulter®).
The comparison between FCMix and WAD tubes was assessed by Wilcoxon test in the MedCalc software for statistical analysis. Significant P values were values below 0.05.
To assess stability, the percentage change (PC%)[(Glucose FCMix – Glucose WAD)/Glucose WAD]*100 was calculated for all samples.
Therefore, reference change values (RCV) were calculated by formula RCV=1.65*CVi/2 for all samples (CVi=5.0%, glucose desirable intra-individual coefficient of variation).
We established a significant difference for glucose when PC% was >RCV (95%CI) which would represent the loss of stability of the magnitude.

RESULTS

Statistically significant differences were observed between both tubes for all samples: basal ($p<0.0001$); 60 min ($p<0.0001$); 120 min ($p=0.0001$); 180 min ($p<0.0001$).
PC% results showed a significant difference for glucose at basal (6.54%) and 180 min (4.45%) leading to the loss of glucose stability in WAD tubes (RCV=4.12%). Glucose at 60 min and 120 min showed PC%<RCV (3.38% and 3.06% respectively).

CONCLUSIONS

This study shows that blood collection tubes containing the additives of citrate, EDTA and fluoride, acting as glycolysis inhibitor, stabilizes glucose concentration leading to an accurate measurement of glucose for the screening and diagnosis of GDM. Based on these results, this tube should be implemented for glucose measurement following the recommendations of the ADA.

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PERFORMANCE EVALUATION OF ABBOTT ALINITY C HBA1C AND COMPARISON WITH BIO-RAD D100 AND ROCHE C502 ASSAYS ACCORDING TO SIGMA METRICS

W. Cao [2], Y. Yang [2], H. Du [2], P. Sun [1], J. Miao [1], C. Chen [3], P. Feng [2]
[1]*China R&D Center, Core Diagnostics, Abbott Laboratories, China*
[2]*Department of Clinical Laboratory, The Second Affiliated Hospital of Soochow University, China*
[3]*Scientific Affairs, Core Diagnostics, Abbott Laboratories, Singapore*

BACKGROUND-AIM

HbA1c is the preferred test in diabetes management and must be accurate and precise to warrant its clinical use. The objective of the study is to evaluate the analytical performance of Alinity c HbA1c and to compare with Bio-Rad D100 and Roche c502 assays using accuracy based Sigma metrics.

METHODS

Analytical performance including precision, accuracy, linearity and method comparison was assessed for Alinity c HbA1c and the comparator assays. The 5-day precision evaluation per CLSI EP15-A3 was performed using Bio-Rad Lyphochek® Diabetes Control and two patient sample pools with HbA1c value at approx. 6.5% and approx. 8%, respectively. Accuracy was evaluated by testing 2 frozen whole blood samples sourced from an IFCC approved reference laboratory in China with HbA1c values assigned by the IFCC measurement procedure. Linearity was assessed using 7 levels of patient sample pools with HbA1c value ranging from 4% to 14%. Method comparison with Bio-Rad D100 and Roche c502 assays was performed per CLSI EP9-A3 using 150 patient samples spanning a measurement range of 4%-14%. Sigma metrics for each assay were determined relative to the total allowable error at 6.9% (in DCCT units) as proposed by the IFCC Task Force.

RESULTS

Within-lab (total) imprecision for Alinity c HbA1c and the 2 comparator assays was all < 2% CV for the Bio-Rad Control and patient pools. Alinity c HbA1c showed a better precision performance in all of the samples tested. Bias of the 3 assays by testing the reference samples was all below 3%. Alinity c HbA1c exhibited a good correlation with Bio-Rad D100 (Slope=0.994, r=0.998) and Roche c502 (Slope=1.067, r=0.997). Linearity of Alinity c HbA1c was validated for the range of 4.4%-13.3% (r>0.99). Using a relative six sigma performance score, Alinity c HbA1c demonstrated a better score of 4.79 (for low level specimen) and 8.52 (for high level specimen), satisfying the requirement set forth by the IFCC Task Force for both routine testing and clinical trial utilities.

CONCLUSIONS

Alinity c HbA1c assay is a precise and accurate method with a good sigma performance score. It demonstrates suitability for the diagnosis and monitoring of diabetes mellitus.

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RELATIONSHIP OF HEMOSTATIC ALTERATIONS AND LIVER FAT IN PREDIABETES

S. Hörber [3], R. Lehmann [3], N. Stefan [2], A. Königsrainer [1], J. Machann [4], M. Heni [3], A. Fritsche [2], A. Peter [3]

[1]*Department of General, Visceral and Transplant Surgery, University Hospital Tübingen, Germany*

[2]*Department of Internal Medicine IV, Division of Diabetology, Endocrinology and Nephrology, University Hospital of Tübingen, Germany*

[3]*Institute for Clinical Chemistry and Pathobiochemistry, Department for Diagnostic Laboratory Medicine, University Hospital Tübingen, Germany*

[4]*Section on Experimental Radiology, Department of Radiology, University Hospital Tübingen, Germany*

BACKGROUND-AIM

Obesity and insulin resistance are associated with an increased risk for arterial and venous thrombosis due to a prothrombotic state which can be improved by lifestyle intervention. Recently we could demonstrate that body fat distribution and liver fat content are closely linked to alterations of pro- and anticoagulant proteins in prediabetes. However, the underlying mechanisms and the sum of all effects on the entire hemostasis system are unclear.

METHODS

100 subjects with impaired glucose tolerance and/or impaired fasting glucose were metabolically characterized including determination of liver fat content using magnetic resonance spectroscopy and performance of an oral glucose tolerance test. Furthermore, the endogenous thrombin potential was determined before and after a 1-year lifestyle intervention. Additionally, gene expression profiles were analyzed in liver tissue samples from 170 independent patients.

RESULTS

Subjects with fatty liver showed increased activities of FVII, FVIII, protein C and protein S, and increased concentrations of fibrinogen. In contrast, control subjects showed higher antithrombin activity. At the molecular level, increased transcription levels of genes encoding for procoagulant proteins (FII, FVII, FIX) as well as anticoagulant proteins (protein C and protein S) were found to be significantly associated with liver triglyceride content.

CONCLUSIONS

Liver fat is closely linked to hemostatic alterations. Subjects with fatty liver showed increased levels of procoagulant and anticoagulant proteins which was confirmed, at least partially, at the molecular level. Further analyses will focus on upstream regulators of hemostasis parameters in relation to liver triglyceride content and include results of endogenous thrombin potential during lifestyle intervention.

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IMPACT OF COVID-19 LOCKDOWN ON OTHER CURRENTS PANDEMICS, A HEAVY DEBT TO BE PAID?

M. Arnaldos-Carrillo [2], C. Pérez-Gomariz [1], I. Cebreiros-López [2], M. Expósito-Garcia [2], M. Navarro-Rey [2], S. Del Pozo-Luengo [2], A.M. Martínez-López De Castro [2], T. Casas-Pina [2], P.L. Tornel-Osorio [2], L. Rosado-Jimenez [2], J.A. Noguera-Velasco [2]

[1]*Clinical Biochemistry Area, Chemistry Faculty, Murcia University, Murcia*

[2]*Clinical Laboratory Service, Clinical Universitary Hospital Virgen de la Arrixaca, Servicio Murciano de Salud, Murcia*

BACKGROUND-AIM

Obesity and metabolic syndrome are also considered to be other pandemics of the 21st century, chronic metabolic diseases that cause high morbidity, mortality and social cost, especially in developed countries. At present, the COVID-19 pandemic and the health measures taken to try to curb the spread of SARS-CoV-2 may have contributed to the aggravation of these types of metabolic disorders. Since it is not known for certain how the lockdown and the COVID-19 pandemic have influenced health habits and the monitoring of chronic diseases, it is hypothesized that this period of confinement and restriction of movement is associated with a worsening of the control in the monitoring of these diseases, which, together with the undeniable medical disregard for all non-covid pathologies, could result in a worse clinical evolution, and even a greater susceptibility to the most severe types of COVID-19.
We analyze the demand of medical laboratory tests between 2019 and 2020 in the management of Diabetes type 2 (DM2) and dyslipidaemia and we compare the values in the prepandemic and confinement and post-confinement periods in the population of a Mediterranean Spanish region

METHODS

The study design is single-center, retrospective, observational, of an ecological nature, covering the tests carried out in 2019 versus 2020. Hospitalized and urgent care patients were excluded.
We analized the records of 124,538 patients and the biochemical parameters studied were cholesterol (CHOL), HDL-cholesterol (c-HDL) and non-HDL-cholesterol (non-c-HDL), LDL-cholesterol (c-LDL), Triglycerides (TG) and hemoglobin A1c (HbA1C)

RESULTS

The results reflect a decrease (49.3%) in the performance of tests on patients during the months of lockdown compared to the same period of the previous year, this could lead to inadequate follow-up and management of these pathologies due to physical inactivity or poor eating habits inherent to the period of confinement and medical neglect caused by the saturation of the health system.
Of all the clinical variables analyzed, the only one for which clinical significance was found was HbA1C during the lockdown period. This result seems to suggest a poor control of DM2 during this period of time.

CONCLUSIONS

it is not possible to confirm that the LOCKDOWN and the COVID-19 pandemic are linked to a worsening in the control of the key variables in these types of pathologies with the exception of DM2 control.
However, there could be the possibility that the period studied may be too short to observe changes such as those proposed in the initial hypothesis.

Diabetes, obesity, metabolic syndrome

M273

SERUM CARBOXYLATED AND UNDERCARBOXYLATED OSTEOCALCIN ASSOCIATION WITH GLUCOMETABOLIC AND CARDIOVASCULAR RISK MARKERS IN CORONARY ATHEROSCLEROSIS DISEASE: ANALYSIS OF A SYRIAN MALE COHORT

H.E. Shahrour [1]
[1]*Department of Biochemistry and Microbiology, Faculty of Pharmacy, Damascus University, Damascus, Syria*

BACKGROUND-AIM

Cumulating evidence indicates that undercarboxylated osteocalcin (ucOC) functions as a regulatory hormone of glucose metabolism. A new line of enquiry between osteocalcin and vascular calcification has emerged in response to observations that the mechanism of vascular calcification resembles that of bone mineralization, thus linking bone and the vasculature. However, studies reported contrasting results about the association between osteocalcin and atherosclerosis. This study was designed to assess potential relationships between different forms of circulating osteocalcin and glucometabolic and cardiovascular risk markers in male patients with coronary atherosclerosis.

METHODS

A cross-sectional study was conducted on 58 male patients, divided according to the severity of coronary artery disease (CAD), determined by coronary angiography assessment: 1. early coronary atherosclerosis (ECA), n=20, patients with mild CAD (<50% stenosis in any major epicardial arteries), and 2. late coronary atherosclerosis (LCA), n=38, patients with severe, multivessel CAD (>50% stenosis in at least one or more major epicardial arteries). The healthy control (HC) group included 26 healthy male subjects. Carboxylated (cOC) and ucOC were measured using ELISA technique.

RESULTS

We observed a significantly lower ucOC levels in both CVD (ECA and LCA) groups than in HC (2.34±2.23 and 2.48±1.60 vs 6.65±1.78 ng/mL, P<0.01). ucOC was inversely correlated with increasing number of cardiovascular risk factors (CVRFs). Moreover, ucOC levels were markedly reduced in high-fasting plasma glucose (FPG) groups (IFG and T2DM-threshold level), compared to normal FPG group. cOC was higher in the IFG group, possibly predicting such condition.

CONCLUSIONS

In the present study, patients with coronary atherosclerosis showed lower ucOC levels, which were inversely correlated with increasing number of CVRFs. Moreover, ucOC levels were markedly reduced in high-FPG groups. Serum ucOC is a potential biomarker for coronary atherosclerosis disease and its measurement may help to establish preventive and therapeutic approaches.

Diabetes, obesity, metabolic syndrome

M274

PERFORMANCE EVALUATION OF THE NEW SIEMENS ANALYZER, ATELLICA DCA FOR POINT OF CARE HBA1C MEASUREMENT

E. Miler [1], E. English [2], G. John [1]
[1]*Department of Clinical Biochemistry, Norfolk and Norwich University Hospital NHS Foundation Trust, Norwich.*
[2]*Faculty of Medicine and Health, University of East Anglia, Norwich, UK.*

BACKGROUND-AIM

The SARS-CoV-2 global pandemic has highlighted the importance for patients in the community to have access to point of care testing (POCT) that have equivalent levels of precision and accuracy to laboratory based methods. With this in mind, we undertook a review of a quantitative latex agglutination inhibition method for HbA1c on a POCT device that is not yet commercially available – the Siemens Atellica® DCA Analyzer.

METHODS

Over the course of a month, we used Clinical and Laboratory Standards Institute (CLSI)-based protocols to assess imprecision (EP05), accuracy (EP09), and linearity (EP06). Sixty-two whole blood EDTA samples, with HbA1c ranging from 20-124 mmol/mol, were measured on both the Siemens Atellica® DCA Analyzer and the A.Menarini Diagnostics Premier Hb9210 analyser (boronate affinity high performance liquid chromatography) at the Norfolk & Norwich University Hospital. To assess imprecision, three patient pools (low (L), medium (M), and high (H)) were run in duplicate twice per day for 21 non-consecutive days. To evaluate linearity, we took two samples (one low and one high HbA1c level) and mixed them together incrementally to produce a range of five levels. EP Evaluator v12 and Microsoft Excel v14 were used for statistical analyses.

RESULTS

The results from the Atellica® DCA correlated well with the A.Menarini Hb9210 analyser with an EP09 Deming regression slope of 1.004 (95% CI 0.977 – 1.030) and an intercept of -1.9 (95% CI -3.8 – 0.0). We observed good within run, between run, and total precision for each patient pool (mean [mmol/mol]: L 35.68; M 68.60; H 102.65; total CV [%]: L 3.1; M 2.1; H 2.5). The results obtained for the EP06 linearity study showed acceptable performance ($p=0.602$; [acceptable if $p \geq 0.05$]).

CONCLUSIONS

Our findings need to be confirmed in a clinical environment with multiple users, rather than in a laboratory setting by scientifically trained personnel. The data indicate that the Atellica® DCA HbA1c assay is comparable to the A.Menarini glycated haemoglobin method currently used in laboratory practice, and is suitable for clinical application of diagnosis and monitoring of diabetes mellitus.

Diabetes, obesity, metabolic syndrome

M275

ASSOCIATION BETWEEN QUALITY OF LIFE AND COMMON METABOLIC BIOMARKERS IN OBESE PATIENTS

I. Mitu [1], C. Preda [1], D. Dimitriu [1], O. Mitu [1], C. Ciobanu [1], G. Bordeianu [1], R. Stanescu [1], M. Ciocoiu [1]
[1]*University of Medicine and Pharmacy "Gr. T. Popa" Iasi*

BACKGROUND-AIM

Few studies describe the relationship between metabolic disease and quality of life (QoL) and analyze the impact of the change in specific metabolic biomarkers on the QoL of the patient. This study evaluates the association between common biomarkers related to metabolic syndrome and the quality of life in obese patients.

METHODS

A study of 49 subjects (aged 35-75 years) with adiposity (fat mass = FM % greater than 23.5% for men and 29.2% for women) was conducted. Body composition was measured by dual-energy x-ray absorptiometry (DEXA) and the metabolic profile was characterized by blood samples. Participants also completed the 36-item Short Form Health Survey Questionnaire (SF-36).

RESULTS

Patients presented the following values: BMI 33.4±5.61 kg/m2, FM% 41.49±6.18, glucose 113.18±34.45 mg/dl, cholesterol 204.8±43.31 mg/dl, triglycerides 172.84±116.78 mg/dl, LDL-c 131.23±45 mg/dl, HDL-c 52.06±12.86 mg/dl, non-HDL-c 154.81±41.89 mg/dl. A statistically significant association was found between glucose and 3 scales of the SF-36 survey: physical function (r=-0.316, p=0.042), role limitations due to physical health (r=-0.356, p=0.021), general health (r=-0.359, p=0.020). Results with a positive association were obtained for cholesterol, LDL-c and non-HDL, including also 2 other scales from SF-36 (role limitations due to emotional problems, social functioning). The results suggest that obese patients with dyslipidemia have a better QoL. The strongest association was found between LDL-c and role limitations due to physical health (r=0.592, p<0.01). The results for triglycerides and HDL-c were not statistically significant.

CONCLUSIONS

Obese patients with high glucose levels associate an impaired QoL from a physical and general health point of view, while obese patients with dyslipidemia associate a better QoL with improvements in social and physical functioning, but also emotional problems.

Diabetes, obesity, metabolic syndrome

M276

PREVALENCE OF GESTATIONAL DIABETES AND DIAGNOSTIC ACCURACY OF O'SULLIVAN TEST IN OUR HOSPITAL

E. Lepe-Balsalobre [1], J. Montenegro-Martinez [1], D. NuÑez-Jurado [1], J.M. Guerrero-Montavez [1]
[1]*Virgen del Rocio University Hospital, Sevilla.*

BACKGROUND-AIM

Gestational Diabetes (GD) is the most frequent metabolic disease associated with pregnancy, with a prevalence between 2-15%. For GD screening, the O'Sullivan test is performed between gestation week 24 and 28, by ingesting 50 grams of glucose. A blood glucose result >140 mg/dL at 60 minutes requires confirmation by Oral Glucose Tolerance Test (OGTT), being necessary that 2 or more points of OGTT exceed the cut-off point established (Glu0<105 mg/dL, Glu60<190 mg/dL, Glu120<165 mg/dL, Glu180<145 mg/dL) to establish the diagnosis of GD. Factors such as maternal age over 30 years, obesity, family history of diabetes, personal history of prediabetes and polycystic ovary syndrome determine a higher incidence of this pathology. The aim of this study was to estimate the prevalence of GD and diagnostic precision of the O'Sullivan test in our hospital.

METHODS

Descriptive and retrospective study in which GD screenings were evaluated for one year (January 2020 to December 2020). Glucose determinations were carried out on the Cobas 8000 analyzer (Roche Diagnostics, Switzerland), using the hexokinase enzymatic method and measuring the amount of NADH formed by photometry. Statistical analysis was performed using SPSS v.19 software (SPSS, Inc., USA).

RESULTS

During the analyzed period, 1403 GD screenings were performed. 1018 (72.5%) presented a negative result and 385 (27.5%) a positive result. The positives were confirmed by OGTT, obtaining 74 diagnoses of GD (20.6%) and 286 false positives (79.4%). The mean age of our population was 33.2 years, while the mean age of the pregnant women diagnosed with GD was 35.7 years.

CONCLUSIONS

- Prevalence of GD in our health area during the year analyzed was 5.3%, according to other similar studies (2-15%).
- Age factor shows its influence on the development of GD, so it should be considered when we interpret the results of the screening test together with a correct anamnesis, in order to identify pregnant women most susceptible to developing this pathology and possible complications.
- O'Sullivan test has a low specificity in the diagnosis of GD, which implies a delay in the diagnosis and an increase in healthcare costs.

Diabetes, obesity, metabolic syndrome

M277

CONCORDANCE OF HBA1C MEASUREMENT USING TWO DETERMINATION METHODS: ENZYMATIC AND IMMUNOTURBIDIMETRIC.

P. Nogueira Salgueiro [1], M. Lorenzo Medina [2], A. Martin Aguila [2], J..J. Gordillo Perdomo [2], L. Ruiz Garcia [2], J.R. Ojeda Ramos [2], M. Fernandez Tagarro [2], C. Dominguez Cabrera [2]

[1] *Clinical Analysis laboratory , University Hospital of Gran Canaria Doctor Negrin , Las Palmas de Gran Canaria*

[2] *Clinical Analysis laboratory , University Hospital of Gran Canaria Doctor Negrin , Las Palmas de Gran Canaria*

BACKGROUND-AIM

Glycosylated hemoglobin (HbA1c) is usually used to monitor the efficacy of diabetes treatment and glycemic control in patients with diabetes Mellitus (DM), as it reflects blood glucose levels within the last 3 months. Currently, with the advent of the recommendations by the Clinical Practice Guidelines, it is also used for the diagnosis of prediabetes stage To verify the reproducibility and concordance of HbA1c measurement using two determination methods: enzymatic and immunoturbidimetric.

METHODS

1,199 samples obtained from patients with suspected or confirmed DM were analyzed using 2 methodologies: Cobas 8000 Modular analyzer (Roche Diagnostics ®) and Alinity c1-series (Abbott Diagnostics ®); through the Accelerator a3600 (Abbott ®) automation system.
R Core Team 2020, version 4.0.2, was used for the statistical study. Lin's concordance correlation coefficient (CCCLin).

RESULTS

Lin's concordance correlation coefficient was calculated: 1199 samples are analyzed have a CCCLin >0,98 .Of this on prediabetes range (HbA1c = 5.7-6.4%) are 458 patientes aboaut CCCLin >0,95 , 591 patientes with DM (HbA1c ≥ 6.5 – 8%) have a CCCLin >0,99 and 150 patientes Hb1Ac levels > 8% have a CCClin >0,97.
The calculation of the coefficient of variation (CV) was 0.3% , between both methods.

CONCLUSIONS

There is a good correlation between the two methodologies, being interchangeable and superimposable, to be used in routine clinical practice and they could be considered as equivalent.
Our findings support the criteria of the International Federation of Clinical Chemistry (IFCC) recommendations as well as the National Glycohemoglobin Standardization Program (NGSP) recommendations which propose that the interlaboratory coefficient of variation should be lower than 3 to 4% for this determination , in our case 0.3%.

Diabetes, obesity, metabolic syndrome

M278

THE INCIDENCE OF GESTATIONAL DIABETES USING THE DIFFERENT DIAGNOSTIC CRITERIA THAT INCLUDES AN ORAL GLUCOSE TOLERANCE TEST

P. Nogueira Salgueiro [1], P.J. Marquez Gonzalez [1], A. Martin Aguila [1], J.J. Gordillo Perdomo [1], L.E. Ruiz Garcia [1], C. Dominguez Cabrera [1], M.M. Lorenzo Medina [1], M. Casado Puente [1]
[1]*Biochemistry , Department of Laboratory Medicine , Doctor Negrin University Hospital , Las palmas de gran canaria*

BACKGROUND-AIM

Gestational Diabetes (GDM) is an alteration of carbohydrate metabolism with a prevalence of 7% of all pregnancies in our country.
Universal screening is performed in pregnant women between 24-28 weeks of gestation. In high risk pregnant women, it is also performed in the first trimester of pregnancy.
This screening consist in the O'Sullivan (50g of glucose) test is performed, if this test is positive (glucose ≥140 mg/dL per hour), a confirmation test is carried out by administering 100g of oral glucose (oral glucose tolerance test, OGTT). GDM diagnosis can be made on the basis of different scientific societies criteria.
The criteria recommended by the Spanish Group on Diabetes and Pregnancy society (GEDE 2020), promoted by the National Diabetes Date Group (NDDG), the reference values of OGTT(100g) ,basal glycemia ≤105 mg/dL , glycemia 1h ≤190 mg/dL , glycemia 2h ≤165 mg/dL, glycemia3h ≤ 145mg/dL, if exist ≥2 points is a positive result, the patient are diagnosed of GDM, this is the most commonly used in our country.
Additionally, Carpenter-Coustan ,the reference values of OGTT(100g) basal glycemia ≤95 mg/dL, glycemia 1h ≤180 mg/dL,glycemia 2h ≤155 mg/dL, glycemia3h ≤ 140mg/dl, if exist ≥2 points is a positive result and the ADA (2011), the reference values of OGTT(100g) basal glycemia ≤95 mg/dL, glycemia 1h ≤180 mg/dL ,glycemia 2h ≤155 mg/ if exist ≥1 points is a positive result.
To compare the incidence of GDM in a sample of 1,006 patients using the different diagnostic criteria that includes an oral glucose tolerance test.

METHODS

Data was collected from the Laboratory Information System (SmartLis ®). 1,006 reports were reviewed, corresponding to OGTT curves made from June 2019 to March 2020.

RESULTS

Of 1006 OGTT curves ;129 (12,8%) are positive about criterie NDDG , 187 (18,6%) positive about criterie Carpenter-Coustan and 379 (27,8%) criterie ADA2011.

CONCLUSIONS

The use of a less restrictive threshold implies an increase in the prevalence of GDM.
This overdiagnosis could increase non-desired effects in pregnant women, such as anxiety, impairing the correct use of healthcare resources without a significant reduction of adverse effects during the pregnancy.

Diabetes, obesity, metabolic syndrome

M279

CHANGES OF VITAMIN B12 LEVELS IN TYPE 2 DIABETES MELLITUS TREATED WITH METFORMIN

S. Nikolic [1], B. Ilincic [1], V. Cabarkapa [1], D. Benc [1], D. Tomic Naglic [1]
[1]*Clinical Center of Vojvodina, Faculty of Medicine, University of Novi Sad, Serbia*

BACKGROUND-AIM

Metformin represents the first line of medication therapy for type 2 diabetes mellitus (T2DM). In about 10-30% of cases, continuous use of metformin causes impared intestinal absorption of vitamin B12. The exact pathophysiological mechanism leading to metformin induced decreation of vitamin B12 has not been fully known, and there are several theories that attempt to clarify this complex problem.
The aim of this study was to determine the level, dynamics and trend of changes in total vitamin B12 (tB12), holotranscobalamin (B12 active) and homocysteine levels in the blood stream during continuous use of metformin, over a period of one year.

METHODS

The study was carried out at the Center of Laboratory Medicine in cooperation with the Clinic for Endocrinology, Diabetes and Metabolic Diseases, Clinical Center of Vojvodina. This study included 50 T2DM patients at the time of the introduction of metformin therapy. Levels of tB12, B12 active and homocysteine were determined before and after 4, 8 and 12 months of metformin administration, to all subjects.

RESULTS

After a year of metformin use, levels of tB12 has been reduced for 25.3%, and holotranscobalamin for 23.3%. During the study continuous decrease of both, tB12 (mean±SD: 266.3±104.3; 225.5±73.0; 207.1±68.9; 192.4±63.6 pmol/L, p=0.000) and B12 active (mean±SD: 94.1±49.9; 81.6±42.6; 75.1±43.2; 67.6±35.6 pmol/L, p=0.000) levels was observed. Also, a continuous elevation of homocysteine levels was determined, with statistically significant increase in homocysteine values after eight months of metformin administration (median and IQR: 9.95 (8.80-12.30 vs 11.25 (8.90-13.50) μmol/L).

CONCLUSIONS

On the basis of the obtained results, it may be suggested necessity of observation total vitamin B12 and homocysteine levels prior to the introduction of metformin in T2DM therapy and after one year thereafter.

Diabetes, obesity, metabolic syndrome

M280

THE EFFECT OF DIET SUPPLEMENTATION WITH FISH-DERIVED EXTRACTS, FISH-DERIVED PROTEIN HYDROLYSATES AND FISH COLLAGEN ON THE GUT MICROBIOME AND MARKERS OF METABOLIC INFLAMMATION IN A MODEL OF DIET-INDUCED OBESITY

M. Daskalaki [1], K. Axarlis [1], S. Michailidou [3], O. Kolliniati [1], I. Lapi [1], E. Dermitzaki [1], M. Venihaki [1], K. Kousoulaki [2], A. Argiriou [3], Z. El Marsni [4], C. Tsatsanis [1]

[1]*Department of Clinical Chemistry, Medical School, University of Crete, Greece*
[2]*Department of Nutrition and Feed Technology, Nofima AS, 5141 Bergen, Norway*
[3]*Institute of Applied Biosciences (INAB), CERTH, Thermi-Thessaloniki GR -57001, Greece*
[4]*Seagarden AS, Karmsund Fiskerihavn, Husøyvegen 278, 4262 Avaldsnes , Norway*

BACKGROUND-AIM

Obesity and insulin resistance are central traits of the metabolic syndrome and contribute to serious pathologies, such as type 2 diabetes, hypertension and cardiovascular disease. It has been recently highlighted that the gut microbiome contributes to the development of obesity and insulin resistance and its modulation can ameliorate related pathologies. Aim of the work was to determine the impact of dietary supplementation with fish-derived extracts and fish derived protein hydrolysates on the gut microbiome composition and markers of obesity and metabolic inflammation.

METHODS

Fish-derived extracts and fish-derived protein hydrolysates protein hydrolysates including collagen were used as nutritional supplements in the model high-fat induced obesity in mice. We aimed to test microbiome modulation using the aforementioned nutritional supplements through the progression and establishment of the obese phenotype using a 4-week and an 8-week high-fat diet administration protocol. Microbiome isolation followed the termination of the protocol.

RESULTS

Diet supplementation with fish-derived extracts exhibited reduced insulin resistance independent of the weight they gained while fish extracts and fish protein hydrolysates that contained collagen induced beneficial changes in the gut microbiome. Insulin resistance was suppressed at the early stages of obesity. No effect was observed in pro-inflammatory cytokines and chemokines that were analysed.

CONCLUSIONS

Fish-derived extracts and protein hydrolysates suppress type 2 diabetes and this action was be partly due to collagen-induced modulation of the gut microbiome.

Work was supported by the EU Commission and BBI-JU Horizon H2020, through the AQUABIOPRO-FIT project grant number 790956 and the research project “Center for the study and sustainable exploitation of Marine Biological Resources” (CMBR, MIS 5002670) in the framework of the National Roadmap for Research Infrastructures.

Diabetes, obesity, metabolic syndrome

M281

ATELLICA DCA HEMOGLOBIN A1C ASSAY IS NOT AFFECTED BY THE HEMOGLOBIN J VARIANT

J. Mayfield [1], M. Lynch [1], J. Stradinger [1]
[1]*Siemens Healthineers*

BACKGROUND-AIM

Glycation of the N-terminal residue of the beta domain of hemoglobin (Hb) generates hemoglobin A1c (HbA1c). The quantitative measurement of HbA1c is reflective of blood glucose levels over time. There are several well-established techniques for determining the concentration of HbA1c present in a blood sample based on its biophysical properties. Genetic mutations in Hb often result in amino acid substitutions at various positions in the molecule, altering those properties. The species that result from these mutants are known as Hb variants and have the potential to disrupt HbA1c analytical techniques, yielding inaccurate results. The hemoglobin J (HbJ) variant is typically characterized by the removal or addition of a charged residue, and several HbJ variants exist. This type of mutation can lead to changes in Hb surface charge and disrupt methods that rely on charge separation, including electrophoresis and HPLC techniques. These HbJ variants would not be expected to affect results from immunoassay formats such as the Atellica® DCA HbA1c point-of-care system. To confirm this, clinical samples with the HbJ variant were tested on the Atellica® DCA Analyzer*.

METHODS

Fifteen samples with known levels of HbJ variant were obtained from a European Union Reference Laboratory and tested on four Atellica® DCA systems. Samples were determined to contain ~50% variant by capillary electrophoresis and were assigned HbA1c values using the Trinity Biotech PREMIER Hb9210 analyzer boronate affinity method.

RESULTS

Eleven samples were tested with a mean value of 6% HbA1c, which resulted in a mean bias of 1.9% after adjustment for bias to the Trinity Biotech PREMIER system. Similarly, four samples with 8% HbA1c show a bias of 1.8% after normalization.

CONCLUSIONS

This demonstrates that the presence of HbJ (up to 50%) results in <2% bias at normal (<6.5% HbA1c) and abnormal (>6.5% HbA1c) levels using the Siemens Healthineers Atellica® DCA HbA1c assay compared to a reference device when normalized for HbAA bias between the devices. These results suggest that the Atellica® DCA HbA1c assay is unaffected by these HbJ genetic variants, and results obtained with this system can be used to monitor glycemic control in patients carrying the HbJ variant.
*Not available for sale in the U.S.

Diabetes, obesity, metabolic syndrome

M282

EVALUATING THE EFFECT OF FREE GLYCEROL FOR SERUM TRIGLYCERIDES MEASUREMENT IN TYPE 2 DIABETIC PATIENTS.

E. Rufo Vicente [1], J. Rodríguez Domínguez [1], M.A. García Martín [1], J.A. Parejo [1], M. Martínez-Bujidos [1], C. Fernández-Prendes [1]
[1]*Hospital Universitari Germans Trias i Pujol, Spain*

BACKGROUND-AIM

The accurate and precise measurement of triglycerides (TG) is important due to the adverse effects associated with hypertriglyceridemia. Most chemistry analyzers methods are based on enzymatic hydrolysis of TG followed by total glycerol measurement.
In insulin-resistant states, increased lipolysis leads to glycerol and free fatty acids overproduction. An elevated free glycerol concentration may result in the overestimation of TG.
The aim of the study was to evaluate free glycerol concentration in type 2 diabetic patients and to assess the effect of free glycerol on serum TG determination.

METHODS

The study was divided into two parts:
- Serum samples were collected from 50 type 2 diabetic patients (age 64±11 years; 38% women) and 50 from control patients (age 48±19 years; 66% women) to measure free glycerol concentration. The comparison of glycerol concentration between groups was assessed by T-student test assuming unequal variances.
- 38 serum samples were randomly collected. Glycophos® was added to obtain a free glycerol concentration of 116.5 [88-161] µmol/L. TG concentrations before and after adding Glycophos® were measured. Data were compared using paired samples T-student test.
To evaluate the effect of free glycerol on TG determination, percentage change (PC %) and reference change values (RCV) were calculated as follows:
RCV=1.65 x CVi*/2 (95% statistical confidence)*CVi=desirable intra-individual coefficient of variation.
All measurements were performed by AU5800 (Beckman Coulter). Statistical analysis was carried out by MedCalc software (significant values $p<0.05$).

RESULTS

T-student showed a significant increment of free glycerol ($p<0.001$) in type 2 diabetic patients (mean=117.72 µmol/L) compared with the control group (mean=51.34µmol/L).
Comparison between TG concentration before and after adding Glycophos® showed statistically significant differences ($p<0.001$). However, these differences were not clinically significant PC% < RCV (3.5% < 10%).

CONCLUSIONS

Type 2 diabetic patients have higher free glycerol concentrations than the general population. This can lead to a slight increment in TG results. Nevertheless, these TG increases despite glycerol concentration are not clinically significant.

Diabetes, obesity, metabolic syndrome

M283

EVALUATION OF A NEW METERING ALGORITHM TO ENABLE WHOLE BLOOD SAMPLING FOR VITROS® CHEMISTRY PRODUCTS A1C SLIDES* ON THE ORTHO VITROS® 5600/XT 7600 INTEGRATED AND 4600/XT 3400 CHEMISTRY SYSTEMS

A. Cantrell [1], S. Diol [1], A. Gerstenberger [1], E. Graham [1]
[1]*Ortho Clinical Diagnostics, Rochester, NY*

BACKGROUND-AIM

VITROS Chemistry Products A1c Slides* are intended for the measurement of glycated hemoglobin A1c (a1c). Suspended whole blood samples are required to enable accurate A1c testing on clinical chemistry instruments. This can complicate the lab's workflow. A new metering algorithm as a part of VITROS INTELLICHECK® technology enables whole blood sampling and confirms that adequate red cells are sampled to the VITROS A1c Slides* directly from a collection tube without any pre-treatment steps. The efficacy of the whole blood metering algorithm to resuspend the whole blood cells has been demonstrated for up to 20 minutes.
Many factors can affect the efficacy of the metering algorithm to resuspend whole blood samples including, sample volume, dimension of the collection tube, and inherent qualities of the sample such as erythrocyte sedimentation rate (ESR) and hemoglobin concentration. The instrument protocol to test and mitigate whole blood samples for A1c must be robust to all these factors.

METHODS

The efficacy of whole blood sample resuspension was assessed by computing the percent bias between the VITROS A1c Slides measurement for standard 4 mL EDTA samples allowed up to 20 minutes settling and for fully suspended samples (zero minutes settling). 130 unique patient samples were cumulatively tested on four VITROS Systems (VITROS 5600/XT 7600 Integrated and VITROS 4600/XT 3400 Chemistry Systems). Samples included local draws, sourcing from various vendors, and pre-screened high ESR samples. Samples tested had hemoglobin concentrations between 8 and 17g/dL and %A1c values between 4 and 11%. A sample is considered "passing" if the bias of the partially settled sample's %A1c measurement is within ± 5% of the suspended sample's %A1c measurement.

RESULTS

The bias between the 20 minute partially settled samples and the fully suspended samples was within ± 5% for all 130 EDTA samples and all VITROS Systems tested.

CONCLUSIONS

The data presented here demonstrates that the new metering algorithm is effective for at least 20 minutes sampled out of a collection tube or sample cup. This feature significantly improves the lab's workflow.
*Under development

Diabetes, obesity, metabolic syndrome

M284

EVALUATION OF CONCORDANCE FOR VITROS® CHEMISTRY PRODUCTS A1C SLIDES* ON THE ORTHO VITROS AUTOMATED SOLUTION CONFIGURATION WITH VITROS® 5600/XT 7600 INTEGRATED AND 4600/XT 3400 CHEMISTRY SYSTEMS

A. Cantrell [1], S. Diol [1], A. Gerstenberger [1], E. Graham [1]
[1]*Ortho Clinical Diagnostics, Rochester, NY*

BACKGROUND-AIM

VITROS® Chemistry Products A1c Slides* are intended for the measurement of A1c and are compatible with the VITROS Automation Solution (VAS) track to enable customers to automate testing of whole blood samples. It will de-cap the test tube and route to the VITROS Systems for sample check, testing, and analysis. There is no pre-treatment step. This study demonstrates acceptable testing within the default time-out on VAS. User adjustment of the time-out is possible.

METHODS

The result obtained with the VITROS A1c Slides* measurement on VAS compared to the VITROS 5600/XT 7600 Integrated Systems and VITROS 4600/XT 3400 Chemistry Systems was evaluated for both correlation and precision. Forty (40) patient samples were tested across the VITROS Systems (10 samples per VITROS System) at 10 replicates per fluid-system combination. Two (2) levels of quality control fluids were also tested across each VITROS System at thirty (30) replicates per fluid-system combination. Precision data were analyzed by performing a statistical F-test across each system and quality control fluid combination and across each system and all patient sample replicates at a 95% confidence interval. VITROS System correlation to VAS was analyzed by performing a paired T-test and linear regression across each VITROS System and patient sample means at a 95% confidence interval. Data were additionally summarized by computing the grand mean, SD (SD for patient samples pooled by patient), and %CV.

RESULTS

The F-test conducted for precision assessment showed no statistical difference (p-values > 0.05) at a 95% confidence interval for both patient samples and quality control fluids for each system. The paired T-test conducted across the ten (10) patient sample means showed no statistical difference at a 95% confidence interval for each system. Linear regression analysis showed excellent correlation between System and VAS: VAS = 1.036 * VITROS 4600 A1c - 0.19; (r) = 0.998, VAS = 1.019 * VITROS 5600 A1c - 0.10; (r) = 0.999, VAS = 0.975 * VITROS XT 3400 A1c + 0.138; (r) = 0.994, VAS = 0.961 * VITROS XT 7600 A1c + 0.212; (r) = 0.995.

CONCLUSIONS

The data presented here demonstrates that VITROS A1c Slides* show excellent concordance between testing on VAS and on the VITROS Systems.
*Under development

Diabetes, obesity, metabolic syndrome

M285

DIFFERENCES OF FGF21 LEVELS BETWEEN DIABETICS AND NO DIABETICS AND CORRELATION OF FGF21 WITH LIPID CONCENTRATIONS

M. Trapali [1], A. Danalatos [1], D. Houhoula [2], E. Konsta [1], D. Lantzouraki [1], P. Karkalousos [1]
[1]*Department of Biomedical Sciences, Department of Medical Laboratories, University of West Attica, Egaleo, Athens*
[2]*Department of Food Science and Technology, University of West Attica, Greece, Agiou Spyridonos 28, Egaleo, Attiki*

BACKGROUND-AIM

BACKGROUND : Diabetes mellitus type 2 (DM2) is a disease caused by genetic and environmental factors. DM2 often coexists with dyslipidemia, which increases cardiovascular risk. Fibroblast growth factor 21 (FGF21) has multiple beneficial effects on obesity, T2D, and its complications. In adults, FGF21 was found to be positively associated with obesity, fasting insulin, and triglycerides (TG), and negatively with high density lipoprotein (HDL). Serum FGF21 is also elevated in obese children and is associated with free fatty acid (FFA) and leptin levels, whereas weight loss is accompanied by a decrease in FGF21 levels, indicating that obesity causes elevation in FGF21 concentration. FGF receptors are expressed in pancreatic β cells of adult mice, and dominant-negative mutations of the FGF receptors lead to decreased number of β cells and development of diabetes. Administration of FGF-21 in rodents reduces plasma glucose and triglycerides to near-normal levels and improves insulin sensitivity independent of reduction in body weight and adiposity.
AIM: The study of the differences of FGF21 levels between men and women diabetics and men and women non-diabetics. The study of the correlation between FGF21 and blood glucose, HbA1c, Cholesterol, HDL, LDL and Triglycerides.

METHODS

MATERIAL AND METHODS: We collected randomly 58 venous blood specimens from 35 diabetics and 23 non-diabetics. Thirty of them were women (21 - 96 years old) and twenty-eight were men (18 - 83 years old). Diabetes status was raised by glucose and HBA1c levels. Whole EDTA blood samples were used for the determination of HbA1c with turbidity method and the derived plasma samples were used for the determination of Chol, HDL, LDL, TG and Glucose by a colorimetric assay Kit. FGF21 was measured with the quantitative sandwich enzyme immunoassay kit (Biotech R&D systems).

RESULTS

RESULTS: FGF21 levels are much higher in diabetics (643 ± 53.2 pg/mL) than in normal individuals (277 ± 21.8 pg/mL) with a statistically significant difference ($P < 0,001$). Significant differences were also found between the whole diabetic group and healthy subjects for Glucose, HbA1c, Chol, HDL, LDL and TG ($p<0.05$). However, FGF21 levels in diabetic men and women are independent of the levels of glucose, HbA1c, Chol, TG, HDL, LDL ($R2 < 0,4$). FGF21 levels are higher in women than in men in both normal and diabetic individuals but these differences were not statistically significant ($P > 0,3$)

CONCLUSIONS

CONCLUSIONS: Further studies are necessary to replicate these data taking into consideration the gender dimorphism in lipids metabolism, the body mass index and the age which affects hormone and muscle mass of the individuals.

Diabetes, obesity, metabolic syndrome

M286

EVALUATION OF THE NEW ENZYMATIC VITROS® A1C CHEMISTRY PRODUCTS SLIDES* ON THE ORTHO VITROS 5600/ XT 7600 INTEGRATED AND 4600/XT 3400 CHEMISTRY SYSTEMS

G. Snodgrass [1], M. Barbero [1], T. Dimagno [1], J. Fyles [1], C. Hoffman [1], B. Johnson [1], A. Zeberl [1]
[1]*Ortho Clinical Diagnostics, Rochester, NY 14626*

BACKGROUND-AIM

VITROS® Chemistry Products A1c Slides* are being developed for the measurement of glycated hemoglobin A1c on the VITROS 5600/XT 7600 Integrated and VITROS 4600/XT 3400 Chemistry Systems. The analytical performance of VITROS A1c Slides have been evaluated for method comparison, precision, and interference to common hemoglobin variants. The VITROS A1c Slides* use VITROS MicroSlide dry technology and whole blood that is sampled directly from standard EDTA collection tubes without any pre-treatment step. The VITROS INTELLICHECK® technology confirms that adequate red cells are sampled to deliver accurate results. The VITROS A1c Slides* have a high throughput of approximately 180 tests/hr, are compatible to use with the VITROS Automation Solution track and are anticipated to have ≥ 6 months calibration interval.

METHODS

The total within-lab precision was calculated using an ANOVA across three analyzers each from the VITROS 4600/ XT 3400 Chemistry and 5600/XT 7600 Integrated Systems with whole blood patient pools using two replicates per day, twice per day over 20 days (n=80). Accuracy was determined using EDTA venous whole blood patient samples following CLSI EP09c guidelines on a VITROS 5600 Integrated System (n=134, 28-108 mmol/mol A1c) against the NGSP secondary reference laboratory Tosoh G8 HPLC. Assay performance in samples with common hemoglobin variants HbAC, HbAD, HbAE and HbAS was determined versus the Trinity Primus Ultra2 (HbAC, HbAS) or Trinity Premier (HbAD, HbAE).

RESULTS

The total within lab precision from a single ANOVA across all twelve instruments was less than 3.3% CV for all fluids (mean 32, 51, 67, and 103 mmol/mol A1c; CV 3.3%, 2.3%, 2.3%, and 2.4%). The method comparison study for the VITROS A1c Slides* showed excellent correlation with the Tosoh G8 HPLC A1c method: VITROS A1c = 0.979 * Tosoh A1c + 0.03; (r) = 0.982 on the VITROS 5600 System. Samples containing hemoglobin variants HbAC, HbAD, HbAE, and HbAS show no interference (bias < 7%) when tested with VITROS A1c Slides*.

CONCLUSIONS

The data presented here demonstrate that the new VITROS A1c Slides* on the VITROS Integrated and Chemistry Systems show excellent analytical performance and enhanced ease of use with the benefits of VITROS MicroSlide technology.
*Under development

Diabetes, obesity, metabolic syndrome

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EVALUATION OF ANALYTICAL PERFORMANCE OF AN ASSAY FOR THE MEASUREMENT OF NON-ESTERIFIED FATTY ACIDS

A. Casanovas [1], J. Català-Solsona [1], L. Arroyo [1], R. Perez [1], M. Sanchez [1]
[1]*BioSystems S.A., Barcelona, Spain*

BACKGROUND-AIM

Fatty acids are central components of lipid metabolism that can be oxidised for metabolic energy production or serve as a substrate in the synthesis of structural lipids and signaling molecules. Non-esterified fatty acids (NEFA) are released from adipose tissue by lipolysis and are transported in the blood bound to albumin. Circulating NEFA are taken up by the liver and peripheral tissues and can mainly be used for metabolic energy production, re-esterified to triglycerides and exported as very low density lipoproteins by the liver or used for hepatic synthesis of ketone bodies. The concentration of NEFA in serum is affected by fluctuations of hormones such as insulin and catecholamines. Pathological states such as type 2 diabetes and obesity are associated with increased concentrations of NEFA in serum and contribute to the development of cardiovascular diseases. BioSystems has developed an assay for the measurement of NEFA in serum and plasma samples. The assay is based on enzymatic reactions that generate a coloured product that can be measured by spectrophotometry at 560 nm. The aim of this study is the evaluation of analytical performance of this new assay.

METHODS

The assay has been applied to automated BioSystems analyzers. Detectability, linearity, precision and interferences were evaluated according to the Clinical and Laboratory Standards Institute guidelines. Correlation with a commercially available method was determined by linear regression analysis.

RESULTS

The obtained measuring range of the assay was 0.07 – 4 mmol/L. Total precision (CV) was < 3.5 %. Hemoglobin (200 mg/dL), bilirubin (30 mg/dL), triglycerides (1000 mg/dL) and ascorbic acid (30 mg/dL) did not interfere at the indicated concentrations. High correlation was obtained in the comparative study with serum ($y = 1.01 x - 0.0446$; $r = 1.000$) and plasma ($y = 1.028 x - 0.0477$; $r = 1.000$) samples.

CONCLUSIONS

The BioSystems NEFA assay shows optimal precision, detectability, linearity, specificity and good correlation in the comparison with a commercially available method. The assay has been successfully applied to automated BioSystems analyzers of different throughput. Overall, the analytical performance of the assay demonstrates its suitability for clinical use.

Diabetes, obesity, metabolic syndrome

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PREVALENCE OF METABOLIC SYNDROME IN STUDENTS OF THE MEDICAL FACULTY IN FOčA

D. Puhalo Sladoje [1], D. Bokonjic [1], A. Mandic [1], O. Cancar [1], V. Cancar [1]
[1]*Faculty of Medicine Foca, University of East Sarajevo, Foca, Republic of Srpska, B&H*

BACKGROUND-AIM

In the modern world, obesity is a major health problem, both among adults and among children and adolescents. Obesity is a disease characterized by abnormal or increased accumulation of fat in adipose tissue to the extent that it damages health and leads to the development of numerous complications. The appearance of obesity at a younger age increases the risk of early onset of these complications, which requires its timely diagnosis and treatment. The aim of this study was to determine the presence of metabolic syndrome in students of the Medical Faculty in Foča.

METHODS

In accordance with the set goals, the research was conducted according to the type of cross - sectional study. In the first part of the examination, a systematic examination of first and second year students of the Medical Faculty in Foča, University of East Sarajevo was performed. The study included 175 subjects, both sexes, aged 19 to 21 years.
As part of the systematic examination, each subject underwent measurement of systolic and diastolic blood pressure (mmHg), measurement of body weight (kg), body height (cm), waist circumference (cm), hip circumference (cm) and based on the obtained results was calculated body mass index (BMI, kg / m2) and waist circumference (cm) / hip circumference (cm) (OS / OK). Laboratory testing included: determination of total cholesterol concentration (mmol / L), cholesterol concentration in HDL (mmol / L), LDL (mmol / L) and VLDL (mmol / L) lipoproteins, apolipoprotein A-1 and B concentration (g / L) and serum triacylglycerol concentration (mmol / L). Sugar metabolism was monitored by measuring blood glucose concentration (mmol / L).

RESULTS

Based on anthropometric parameters and biochemical indicators, and in accordance with the criteria of the International Diabetes Mellitus Association, the mentioned group of subjects was divided into 3 subgroups: the first group - normally fed subjects (N 106), the second group - subjects with abdominal obesity but without metabolic syndrome (N 37) and the third group - subjects with metabolic syndrome (N 32).
Of the total number of subjects included in the study, 69 were found to have abdominal obesity, according to IDF criteria. Among the subjects with abdominal obesity (N 69), in thirty - seven (N 37) no signs of metabolic syndrome were found, while 32 subjects had two components of metabolic syndrome. In the group with abdominal obesity, there were significantly more female subjects ($p < 0.001$). In female subjects, the most common components of the metabolic syndrome were higher waist circumference and decreased HDL-cholesterol concentration, while in men, higher waist circumference, increased triacylglycerol concentration and increased blood pressure were found. Decreased HDL-cholesterol concentration was measured in 93% of the female population of our study group, and among the male population this percentage was 35%.
The prevalence of arterial hypertension in the group with metabolic syndrome was 53.0%
During our study, in the blood of subjects of all examined groups, fasting glycemic values were within the reference values of the applied methods and did not differ significantly between the examined groups.

CONCLUSIONS

Research indicates that the finding of metabolic syndrome components in the population of children and adolescents increases the incidence of fatal and non-fatal cardiovascular events in later years. Therefore, early identification and treatment of children and adolescents with metabolic syndrome and taking preventive measures is crucial to reduce the risk of cardiovascular and other obesity complications.

Diabetes, obesity, metabolic syndrome

M289

EVALUATION OF HEMATOCRIT BIAS ON BLOOD GLUCOSE MEASUREMENT WITH NINE DIFFERENT PORTABLE GLUCOSE METERS

R. Wijngaard [1], J. Laguna [1], M. Rodríguez-García [1], V. San Segundo [2], R. Muñoz [2], J.L. Bedini [2]
[1]*Biochemistry and Molecular Genetics Department, Biomedical Diagnostic Center, Hospital Clinic, Barcelona, Spain*
[2]*CORE Laboratory, Biochemistry and Molecular Genetics Department, Biomedical Diagnostic Center, Hospital Clinic, Barcelona, Spain*

BACKGROUND-AIM

Self-monitoring blood glucose (SMBG) devices are an essential tool for self-care management of diabetes. The accuracy of the SMBG devices can be influenced by a large number of methodological, environmental and physiologic factors, including abnormal hematocrit (HCT) values. Our objective was to evaluate the influence of the HCT on nine SMBG devices.

METHODS

The study procedure followed the indications of the ISO 15197:2013 for HCT interference testing, with one test strip lot per device. We evaluated different HCT levels within the acceptable HCT range as described in their respective instructions for use. The mid HCT level was established at 42% +/- 2%. The three glucose ranges were: 30-50 mg/dL, 96-144 mg/dL and 280-420 mg/dL. For each HCT-glucose combination, 5 replicates were tested. Acceptable normalized bias was of 10 mg/dL or 10% for glucose <100 mg/dL or >100 mg/dL, respectively.
Reference glucose measurements were performed on an Atellica® Solution (Siemens Healthineers) using hexokinase method. HCT levels were quantified by an Advia® 2120 (Siemens Healthineers).

RESULTS

The highest bias of the three glucose ranges, for each device and HCT, were: CONTOUR® Next One 3.8 at 60% HCT, Accu-Chek® Performa 14.7 at 65% HCT, Accu-Chek® Aviva 12.9 at 65% HCT, GlucoMen® Areo 18.8 at 70% HCT, FreeStyle Lite 20.0% at 15% HCT, FreeStyle Optium Neo -5.5% at 30% HCT, FreeStyle Libre 13.1 at 30% HCT, OneTouch Verio Reflect® -9.1 at 60% HCT and OneTouch Select Plus® 14.3 at 55% HCT. The hematocrit interference observed in the Contour® Next One, FreeStyle Optium Neo and OneTouch Verio Reflect® fulfilled the ISO 15197:2013 specifications. However, Contour® Next One was able to provide reliable results in a wider hematocrit range and always within 5% of bias.

CONCLUSIONS

Health care professionals and patients should be aware of the SMBG limitations and select the SMBG device that would fit best the individual situation of each patient.

Diabetes, obesity, metabolic syndrome

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EFFECT OF A NOVEL LACTOBACILLUS STRAIN ON SERUM CHOLESTEROL AND METABOLIC ALTERATIONS IN A MODEL OF HIGH FAT DIET INDUCED OBESITY

E. Ieronymaki [1], V. Santarmaki [3], E. Dermitzaki [1], P. Panas [4], A. Argiriou [2], Y. Kourkoutas [3], C. Tsatsanis [1]
[1]*Department of Clinical Chemistry, Medical School, University of Crete, Greece*
[2]*Institute of Applied Biosciences (INAB), CERTH, Thermi-Thessaloniki, Greece*
[3]*Laboratory of Applied Microbiology and Biotechnology, Department of Molecular Biology & Genetics, Democritus University of Thrace, Greece*
[4]*QLCon, Patras, Greece*

BACKGROUND-AIM

Probiotics have been associated with intestinal and organism homeostasis and when administered in adequate amounts alter the gut microbiome and confer a health benefit to the host. Gut microbiome is an important contributor to the pathogenesis of obesity and related diseases. Studies have shown that probiotics, including Lactobacillus and Bifidobacterium species, suppress adiposity and low grade inflammation associated with obesity. Aim of this study is to determine the impact of a novel probiotic strain of the Lactobacilus family on markers of obesity.

METHODS

We have isolated a novel strain of Lactobacillus, a probiotic known to be beneficial in human health. In order to study the effect of the selected probiotic in obesity, we utilized a mouse model of obesity. C57BL/6 mice were fed a normal or high fat diet (HFD) supplemented or not with free or immobilized probiotic for eight weeks. Following an in vitro screening and in vivo testing of a series of probiotics we selected a wild strain of Lactobacillus, which was administered daily at a concentration of 109cfu/g. Insulin resistance was evaluated using Glucose Tolerance Test (GTT). Body and adipose tissue weight were measured. Serum cholesterol levels were also obtained at different time points.

RESULTS

Evaluation of insulin resistance 4 days after initiation of the experiment showed that mice fed a HFD supplemented with Lactobacillus either free or immobilized had not developed insulin resistance compared to HFD fed control mice. Mice fed HFD supplemented with immobilized Lactobacillus demonstrated improved glucose tolerance compared to control HFD fed mice, even after 60 days of feeding, at a stage of advanced obestiy. Serum cholesterol levels were significantly lower in mice fed with HFD supplemented with immobilized Lactobacillus compared with mice fed with HFD supplemented with immobilization support. In accordance, this group also showed reduced epididymal adipose tissue weight. Adipose tissue weight was also significantly lower in mice fed with HFD supplemented with free Lactobacillus compared to mice fed HFD.

CONCLUSIONS

In conclusion, this new Lactobacillus strain suppresses adiposity, dyslipidemia and delays the development of insulin resistance found early in obesity.

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Diabetes, obesity, metabolic syndrome

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GENETIC SCREENING OF MORBID OBESITY IN A FRENCH REFERENCE OBESITY CENTER

D. Collin-Chavagnac [3], B. Segrestin [1], M. Laville [2], E. Disse [2]

[1]*Département Endocrinologie, Diabète et Nutrition, Hospices Civils de Lyon, Hôpital Lyon Sud, Pierre-Bénite France*

[2]*Département Endocrinologie, Diabète et Nutrition, Hospices Civils de Lyon, Hôpital Lyon Sud, Pierre-Bénite France / CarMen Laboratory, INSERM, INRA, INSA Lyon, Université Claude Bernard Lyon 1, Pierre-Bénite France*

[3]*Service de Biochimie et Biologie moléculaire, Hospices Civils de Lyon, Hôpital Lyon Sud, Pierre-Bénite, France*

BACKGROUND-AIM

Obesity is a multifactorial disorder, phenotypically characterized by an excess of body fats, and caused by several environmental and genetic risk factors with the heritability of about 40 -70%. Genetic factors play a crucial role in determining an individual's predisposition to weight gain and being obese. Defects in at least 15 genes are the cause of monogenic obesity cases, resulting mostly from deficiencies in the leptin-melanocortin signaling pathway. We realized a genetic screening in morbid obese patients seeking for obesity management in a reference obesity center.

METHODS

46 consecutive morbid obese patients were enrolled. Beside a precise clinical and biological phenotyping, the population was young and the sex ratio M/F was 0.4. A familial obesity was present in 86.3%. Genomic DNA was extracted from EDTA peripheral blood, libraries were constructed using SeqCap EZ Solution-Based Enrichment strategy (Roche NimbleGen Madison, WI, USA) and sequenced on a NextSeq500 sequencer (Illumina, San Diego, CA, USA), performed with a custom Next Generation Sequencing workflow.

RESULTS

In our cohort no mutation in Melanocortin-4-Receptor (MC4R) gene was highlighted except a complete heterozygous deletion of MC4R gene in one patient. We reported 40 variants in POMC, LEPR, LEP, and INSR genes, in coding sequences or flanking regions. 5 variants were classified as risk factors for obesity considering in silico analysis and literature. We also checked the CNV status of our panel of genes in each patient. We reported 9 CNV including the MC4R CNV.

CONCLUSIONS

We reported several genetic arguments that could explain the obesity phenotype into our cohort of 46 patients. However, we couldn't explain genetically all their severe obese phenotype. This work underlined the heterogeneity and complexity of obesity and its genomic network where multiple pathways were nested. Current knowledge will make it possible to discover the involvement of certain variants/genes and to better understand this pathology. CNV analysis is a criterion that should not be neglected in this pathology. The discovery of new genes involved in different signaling pathways opens the door to many future treatments of obesity such as peptide-based agonists for which genetic analysis will be indispensable.

Diabetes, obesity, metabolic syndrome

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MEASUREMENT OF HBA1C IN PACKED CELLS (RED BLOOD CELLS) USING THE ABBOTT HEMOGLOBIN A1C ASSAY

P. Henriques [1], E. Miler [2], E. English [2], G. John [2]
[1]*Department of Clinical Biochemistry, James Paget University Hospital, Great Yarmouth, UK*
[2]*Department of Clinical Biochemistry, University of East Anglia, Norfolk and Norwich University Hospital, Norwich, UK*

BACKGROUND-AIM

The importance of Biorepositories in medical research is increasingly being recognised. These unique and/or hard to obtain samples have proved invaluable for large scale research studies. Being able to use samples from Biorepositories for the measurement of haemoglobin A1c (HbA1c) would be valuable; but these small volume samples tend to be stored frozen as packed red blood cells (RBC), limiting their usefulness. Thus this study evaluated the utility of determining HbA1c values from packed RBC using a commercial HbA1c assay.

METHODS

A comprehensive evaluation of the Abbott Enzymatic HbA1c method was undertaken using Clinical and Laboratory Standards Institute-based protocols to assess imprecision (EP05), accuracy (EP09), and linearity (EP06). 50 whole blood EDTA samples, with HbA1c ranging from about 20-120 mmol/mol, were measured on both the Abbott Alinity c Analyzer and the A.Menarini Diagnostics Premier Hb9210 analyser (boronate affinity high performance liquid chromatography). To assess imprecision, three patient pools (low, medium, and high) were run in duplicate twice per day for 20 non-consecutive days. To evaluate linearity, two patient samples (one low and one high HbA1c level) were mixed together incrementally to produce a set of panels at five distinct levels. The Abbott Enzymatic HbA1c method was adapted to use a manual pretreatment procedure to measure HbA1c on packed RBC samples before and following storage at -80C.

RESULTS

The Abbott Enzymatic HbA1c method correlated well with the A.Menarini Hb9210 analyser with a regression slope of 1.00 and an r of 0.99. Total imprecision for each patient pool: mean (mmol/mol) was: 35.68; 68.60; 102.65; respective total CV (%): 3.1; 2.1; 2.5. The results obtained for the EP06 linearity study showed acceptable linear performance [acceptable if $p \geq 0.05$]. The modified Abbott enzymatic assay measured 10uL of packed red blood cells when diluted in the assay diluent. No significant difference was found in HbA1c results when frozen packed red cell samples were compared to the original fresh whole blood samples.

CONCLUSIONS

HbA1c can be measured in small packed red cell samples (typically stored in Biorepositories) and the results obtained accurately reflect the results found in fresh whole blood samples.

Diabetes, obesity, metabolic syndrome

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CORRELATION BETWEEN OSTEOPROTEGERIN, ASYMMETRIC DIMETHYLARGININE AND DISEASE DURATION IN PATIENTS WITH LONG-TERM TYPE 1 DIABETES MELLITUS

G. Chausheva [2], Y. Bocheva [2], S. Shefket [2], K. Tsochev [4], V. Iotova [4], Y. Yotov [7], C. Tatyana [7], N. Usheva [5], M. Boyadzhieva [6], G. Valchev [1], R. Pancheva [3]
[1]*Clinic of Diagnostic Imaging, UMHAT "Sv. Marina", MU-Varna, Bulgaria*
[2]*Dept. of Clinical Laboratory, MU-Varna, Bulgaria*
[3]*Dept. of Hygiene and Epidemiology, MU-Varna, Bulgaria*
[4]*Dept. of Pediatrics, MU-Varna, Bulgaria*
[5]*Dept. of Social Medicine and Health Care Organization, MU-Varna, Bulgaria*
[6]*IInd Dept. of Internal Diseases, MU-Varna, Bulgaria*
[7]*Ist Dept. of Internal Diseases, MU-Varna, Bulgaria*

BACKGROUND-AIM

Osteoprotegerin (OPG) and asymmetric dimethylarginine (ADMA) are associated with diabetes mellitus (DM)–related endothelial dysfunction and increased cardiovascular risk. OPG is a soluble glycoprotein, mainly involved in bone metabolism, but also expressed in vascular smooth muscle and endothelial cells. ADMA is an endogenous nitric oxide synthase inhibitor, modulating arterial resistance.
Our aim is to evaluate the correlation between OPG, ADMA and disease duration in long-term type 1 DM (T1DM) patients.

METHODS

The current study period is 2018-2020 year. The studied group included 124 diabetic patients (mean age 42.7 ± 10.4 years; 53.2% male to 46.8% female, without previous cardiovascular event; duration of diabetes 25.3 ± 8.2 years). The control group consisted of 59 age, gender and BMI- matched healthy people (male/female 55.9%/ 44.1%; mean age 45.1 ± 9.1 years). Serum ADMA and OPG levels were determined by enzyme-linked immunosorbent assays (ELISA).

RESULTS

Serum levels of OPG and ADMA were higher in people with T1DM, compared to healthy controls (OPG: 5.53 ± 1.53 to 5.33 ± 1.86 pmol/l, $p = 0.3$; ADMA: 0.56 ± 0.24 μmol/l to 0.55 ± 0.16 μmol/l). A significant positive correlation was observed between the duration of diabetes and OPG levels ($r = 0.22$; $p < 0.05$), unlike ADMA levels. The correlation between OPG levels and ADMA levels in T1DM patients is significant and positive ($r = 0.21$; $p< 0.05$).

CONCLUSIONS

In long-term poorly controlled T1DM patients the correlation between OPG and ADMA and between OPG and the disease duration is positive and significant. According to our results OPG could be established as a better laboratory marker than ADMA for endothelial dysfunction and increased cardiovascular risk, considering disease duration. The latter should be a point of a future research work.
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Diabetes, obesity, metabolic syndrome

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THE EFFECTS OF HIGH-FAT OR HIGH-FAT / HIGH-SUGAR DIETS ON RAT SPERM PARAMETERS AND MITOCHONDRIAL RESPIRATION

N.M. Kozopas [1], B.O. Manko [2], H.M. Mazur [2], H.V. Maksymyuk [1]
[1]*Department of Clinical Laboratory Diagnostics, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine*
[2]*Department of Human and Animal Physiology, Ivan Franko National University of Lviv, Lviv, Ukraine*

BACKGROUND-AIM

A piece of evidence suggests that male obesity negatively affects sperm quality. Presently, the increased sugar consumption is considered the main cause of obesity. The results of few studies indicate that a high-fat diet has a detrimental effect on sperm mitochondrial respiration, yet the effects of additional sugar supplementation are unknown. The aim of this study was to find out if short-term high-fat (HF) and high-fat/high-sugar (HFHS) diets impact the sperm parameters and the mitochondrial respiratory activity in rats.

METHODS

A total of 24 sexually mature white wild-type male rats weighing 250-300g were divided randomly into three groups of 8 animals each: control group (CG) with standard diet, HF diet group, and HFHS diet group. The rats were fed different diets for 7 weeks.
Before the experiment, males were weighed and the nasoanal length was measured. Lee index was calculated. The animals were sacrificed by cervical dislocation. Testes, liver, visceral fat were then removed and weighed.
Caudae epididymides (CE) were excised and were transferred to a Petri dish filled with Hepes-buffered modified Tyrode's medium (mTH) pre-warmed to 37°C. Few cuts were made in each CE using scissors and allowing sperm to swim out for 5-10 min. Sperm count was assessed with a haemocytometer. Rat sperm vitality was assessed with propidium iodide staining using fluorescent microscope Olympus IX73.
Basal and FCCP (protonophore)-stimulated oxygen consumption were determined with Clark electrode. Approximately 20 million of cells suspended in the mTH solution were placed into the respiratory cell. Alternatively, the mTH solution was supplemented with 10 mM lactate.
The difference between groups was assessed by ANOVA with Tukey's test for multiple pairwise comparisons. $P<0.05$ was considered significant.

RESULTS

In three animals from the HFHS group after dissection, we detected neoplastic changes in lungs and liver, in three more cases we failed to isolate living cells. Therefore, we excluded the above cases from the analysis.
We found that the Lee index did not differ significantly between groups (P=0.5855), while the visceral fat coefficient in the HFHS group was 40% higher compared to the CG (P=0.0451). Liver and testis coefficients (ratio to body weight) were unchanged in all groups. No significant difference in sperm count and vitality were found between the study groups (P=0.8261). The proportion of live cells in each group was at least 50%.
The basal respiration rate of spermatozoa in mTH solution without lactate in the HFHS group was 0.123 ± 0.024 nmol O_2/million cells/min, which was 70 % higher than in CG (P=0.0152). A similar effect was observed with a maximal FCCP-stimulated respiration rates – the cells of the HFHS-fed animals consumed oxygen 60% faster (P=0.0139). Adding lactate to the medium changed the rates of basal respiration almost twofold (P=0.0139), yet a significant difference of lactate-driven oxygen consumption was not different between groups (P=0.1522).

CONCLUSIONS

The results of this study show that the short-term HF-diet did not cause obesity in rats and did not significantly affect sperm parameters and mitochondrial respiration. However, 7 weeks of HFHS diet administration caused a substantial increase of visceral fat. In this group, sperm mitochondrial respiratory capacity was slightly better than in controls. We assume that this can be explained by increasing the intensity of protective processes under diet-induced stress. Additional studies are required to elucidate long term effects of this diet on sperm viability and mitochondrial capacity.

Diabetes, obesity, metabolic syndrome

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POSTPRANDIAL INFLAMMATION AND METABOLIC DYSFUNCTION IN ADOLESCENTS WITH OBESITY AND INSULIN RESISTANCE.

S. Wilson [1], V. Higgins [1], K. Adeli [1]

[1]*CALIPER Program, Department of Pediatric Laboratory Medicine, The Hospital for Sick Children; Department of Laboratory Medicine & Pathobiology, Faculty of Medicine, University of Toronto*

BACKGROUND-AIM

Postprandial dyslipidemia is an independent risk factor for cardiovascular disease, and inflammatory and metabolic markers may be implicated in its pathogenesis. We sought to characterize the fasting and postprandial inflammatory and metabolic profiles of adolescents with obesity and insulin resistance (IR), and to assess their role in the pathogenesis of postprandial dyslipidemia.

METHODS

Adolescents with normal weight (N=15) and obesity (N=30) were recruited and underwent a six-hour oral fat tolerance test. Eight cytokines were assessed using an automated immunoassay and a metabolomics profile (>133 metabolites) was quantified using liquid chromatography-tandem mass spectrometry and nuclear magnetic resonance spectroscopy. A lipid/lipoprotein, bile acid, and anthropometric profile were previously quantified in this cohort.

RESULTS

Among the studied cytokines, levels of interleukin (IL)-6 were significantly elevated in obesity throughout the postprandial response ($p<0.05$). Numerous metabolites, namely acylcarnitines, biogenic amines, and phospholipids were significantly downregulated postprandially, whereas amino acids were predominantly elevated, in obesity. Correlational analyses revealed strong (-0.5> Spearman rho >0.5) and significant ($p<0.05$) associations between tumour necrosis factor- α, IL-6, and IL-8 and an atherogenic lipid/lipoprotein phenotype and adiposity and IR, while IL-1ß, IL-18, monokine induced by interferon-γ, and IL-10 were strongly associated with bile acid species. Similarly, acylcarnitines, phospholipids, and biogenic amines were negatively associated with adiposity and IR whereas a positive correlation was observed with bile acids. Biogenic amines, organic acids, and phospholipids were negatively associated with inflammatory markers and positively with hepatic analytes; the opposite was found for acylcarnitines and amino acids. Finally, lipid/lipoproteins were strongly associated with select acylcarnitines and organic acids.

CONCLUSIONS

Adolescents with obesity and IR exhibit significant fasting and postprandial dysregulation of several inflammatory and metabolic markers integral to lipid metabolism. These data may offer novel subclinical biomarkers for early metabolic and cardiovascular diseases, such as postprandial dyslipidemia, in at-risk adolescents.

Diabetes, obesity, metabolic syndrome

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GENE EXPRESSION CHANGES IN ADIPOSE TISSUE IN INDIVIDUALS WITH METABOLIC SYNDROME FOLLOWING LIFESTYLE-INDUCED WEIGHT LOSS

R. Biemann [2], K. Roomp [3], S. Krishnan [2], J.G. Schneider [1], B. Isermann [2]
[1]*Department of Internal Medicine II, Saarland University Medical Center at Homburg/Saar, Homburg, Germany*
[2]*Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig, Leipzig, Germany*
[3]*Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Luxembourg, Luxembourg*

BACKGROUND-AIM

Lifestyle-induced weight loss is regarded as an efficient therapy to reverse metabolic syndrome (MetS) and to prevent disease progression. The aim of this study was to investigate how lifestyle-induced weight loss changes are related to changes in adipose tissue gene expression in individuals with MetS.

METHODS

We analyzed clinical and metabolic changes as well as gene expression of subcutaneous adipose tissue biopsies before and after lifestyle-induced weight loss in 43 well-defined male individuals with MetS in a prospective study by unbiased mRNA profiling (ICTRP Trial Number: U1111-1158-3672).

RESULTS

Lifestyle-induced weight loss was associated with a decline in the BMI (–12.6%) and substantial improvement of insulin sensitivity, systemic low-grade inflammation and lipid metabolism resulting in reversibility of the MetS. Gene expression analysis of adipose tissue biopsies revealed 657 diffenrentially expressed gene probes. Of these, 242 genes were correlated with one or more clinical parameters including weight loss (58.4%), insulin sensitivity (46.9%), serum triglyceride levels (22.2%), cholesterol metabolism (21.0%) or low-grade inflammation (9.9%). Gene set enrichment analysis confirmed cellular cholesterol metabolism as the main pathway altered in subcutaneous adipose tissue following lifestyle-induced weight loss.

CONCLUSIONS

Our results indicate that subcutaneous adipose tissue is a major source for lipid and cholesterol metabolism in MetS. In addition, genes and gene interactions that are potentially metabolically relevant were identified.

Diabetes, obesity, metabolic syndrome

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STUDY OF THE DIAGNOSTIC EFFICACY OF ANTI-ZNT8 ANTIBODIES IN PEDIATRIC PATIENTS FOR TYPE 1 DIABETES

S. Fuentes Cantero [1], R. Galván Toribio [2], C. Rodríguez Chacón [2], F.J. Vázquez Barbero [2], C. González Rodríguez [2], A. León Justel [2]
[1]*Hospital Riotinto*
[2]*Hospital Virgen Macarena*

BACKGROUND-AIM

The zinc transporter 8 (ZnT8) is an islet β-cell secretory granule membrane protein coded by the SLC30A8 gene, identified as a novel autoantigen in human type 1 diabetes (T1D). The most frequently detected autoantibodies in type 1 diabetes mellitus are anti-IA2 antibodies, anti-GAD antibodies and anti-ZnT8 antibodies. Our aim is to study the isolated efficiency of each of these autoantibodies in the diagnosis of type 1 diabetes mellitus in children.

METHODS

For the measurement of Anti-IA2 and Anti-GAD we use the fully-auto Snibe Dcs. chemiluminescence immunoassay (CLIA) automated in the analyzer Maglumi 1000. Anti-ZnT8 were measured by Euroimmun Dcs enzyme-linked immunosorbent assay (ELISA) automated in Euroimmun I2P- analyzer. Statistical analysis in SPSS v25 and statistical significance was considered for $p<0,05$.

RESULTS

A total of 80 patients were studied, 34 boys and 46 girls, of which 50 finally developed diabetes. The median age was 12 years (IQR 5). Of these 50, 34 of them had a family history of diabetes. The results are:
Anti-IA2 antibodies (cut off < 28 U/L): Sensitivity 0,26 (CI95% 0,14-0,38), Specificity 0,97(CI95% 0,79-1,0), LR+ 7,80 (CI95% 1,07-56,66), LR- 0,77 (CI95% 0,63-0,93).
AntiGAD antibodies (cut off <17U/mL): Sensitivity 0,40 (CI95% 0,26-0,54), Specificity 0,87 (CI95% 0,75-0,99), LR+ 3,00 (CI95% 1,13-7,94), LR- 0,69 (CI95% 0,53-0,91).
Anti-ZnT8 antibodies (cut off ≤ 15 U/L): Sensitivity 0,62 (CI95% 0,49-0,75), Specificity 0,97(CI95% 0,90-1,0), LR+ 18,60 (CI95% 2,67-129,33), LR- 0,39 (CI95% 0,27-0,56).
Spearman correlation (p)
Ab antiIA2 vs. Ab antiZnT8: 0,323 (p= 0,004)
Ab antiGAD vs. Ab antiZnT8: 0,503(p= 0,000)

CONCLUSIONS

The results obtained indicate a higher efficiency of anti-ZnT8 antibodies for the diagnosis of diabetes in pediatric patients, despite the small sample size. The correlation between the methods was moderate. Our results indicate the importance of developing diagnostic strategies for this disease that combine the use of these autoantibodies in pediatric patients.

Diabetes, obesity, metabolic syndrome

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COMPARISON OF INSULIN- AND C-PEPTIDE-BASED HOMA2 ESTIMATES OF BETA-CELL FUNCTION AND INSULIN RESISTANCE IN SUBJECTS WITH TYPE 2 DIABETES

E. Matišić [1], M. Krhač Grgić [1], S. Božičević [1], M. Vučić Lovrenčić [1]
[1]*Department of Medical Biochemistry and Laboratory Medicine, Merkur University Hospital, Zagreb, Croatia*

BACKGROUND-AIM

Insulin resistance (IR) and beta-cell function (B) emerged as important tools in differential diagnosis and clinical assessment of the most prevalent type 2 diabetes (T2D). The Homeostasis Model Assessment 2 (HOMA2) calculator provides a practical tool of simultaneous IR and B from fasting plasma glucose and insulin (or c-peptide) values. Considering very short half-life and proteolytic cleveage of plasma insulin, we sought to investigate the performance of the more stable c-peptide in the HOMA2-estimates of IR and B in T2D subjects.

METHODS

Fasting blood samples were taken from 228 subjects (99 females; age range 32-80 yr) with T2D at their regular checkup. The samples were immediately processed and analyses of glucose (hexokinase, Beckman Coulter, U.S.A.) insulin and c-peptide (Advia Centaur XP, Siemens Healthineers, USA), performed within 2 hours from sampling. HOMA2 calculator (https://www.dtu.ox.ac.uk/homacalculator/) was used for the estimation of B and IR index (IRI) from the paired glucose and insulin/c-peptide values, by the use of respective algorithms. The relationships between B and IRI with routinely collected laboratory estimates of diabetes control (HbA_{1C}) and complications (albuminuria, eGFR, total-, HDL-, LDL-cholesterol and triglycerides), were determined.

RESULTS

Insulin- and c-peptide-based estimates of B (I-B and C-B) did not differ (P=0,0723), whereas I-IRI was significantly higher than C-IRI (P=0,0077). At the arbitrary IRI cut-off of 1,4 there were more insulin-resistant subjects with I-IRI than C-IRI (98/228 vs. 86/228; Chi-squared= 104,78; P < 0,0001). However a good concordance between the two IRI estimates (kappa= 0,67389; CI= 0,57687 to 0,77091) in the detection of insulin-resistant subjects was found. Both C-B and I-B were significantly negatively predicted by HbA_{1c} (P<0,0001) and age (P<0,001), while significant negative associations with HDL (P=0,0012; P=0,002) and positive with triglycerides (P=<0,0001; P= 0,0001) were found with C-IRI and I-IRI, respectively.

CONCLUSIONS

In conclusion, insulin- and c-peptide-based HOMA2-estimates of B and IRI performed equally well in T2D patients within a 2h interval from sampling to analysis. C-peptide-based HOMA2 indices may provide more reliable data in laboratory settings with longer turnaround time.

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IMPLICATION OF KLF 14 GENE POLYMORPHISM IN TYPE 2 DIABETES

s. Oueslati [2], M. Zouaoui [2], S. Hadj Fredj [1], A. Guesmi [2], H. Bouhajja [2], M. Dabboussi [2], R. Mahjoub [2], S. Hammami [2], T. Messaoud [1], A. Bibi [2]

[1]*Biochemistry Laboratory, Bechir Hamza Children's Hospital, Tunis, Tunisia.*
[2]*Laboratory of Clinical Biology, National Institute of Nutrition and Food Technology*

BACKGROUND-AIM

Type 2 diabetes (T2DM) is now considered as a major public health problem. It is one of the most frequent pathologies. In fact, global prevalence of T2DM is projected to increase to 7079 individuals per 100,000 by 2030, reflecting a continued rise across all regions of the world. It is currently an important cause of death. There are many serious degenerative complications related to diabetes such as coronary heart disease, kidney failure, and diabetic retinopathy. These complications can be eliminated or at least compensated by adequate treatment. Environmental and genetic factors are involved in the pathogenesis of T2DM. Genome-wide association studies (GWASs), the candidate gene approach, and linkage analysis have identified various genes that contribute to T2DM susceptibility. Therefore, it is possible to facilitate early diagnosis and determine preventative strategies to reduce the incidence of the disease. In our study, we were interested to investigate the association between the rs972283 polymorphism of the KLF14 gene and T2DM in a Tunisian diabetics patients.

METHODS

Our study involved 98 T2DM patients and 95 healthy subjects. The patients as well as the controls studied benefited from an assay of fasting glucose, total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol, creatinine, CRP, hemoglobin A1c (HbA1c), insulin, as well as 25(OH) vitamin D3. Genotyping of the rs972283 polymorphism was performed using the HRM and direct sequencing. Statistical analysis was performed using SPSS software (version 25.0).

RESULTS

The statistical study revealed a significant difference between the two populations in fasting glucose, HbA1c, total cholesterol, triglycerides, CRP, insulin and vitamin D.
There was no significant difference in genotypic distribution between the two populations analyzed. However,a significant difference was observed in the allelic distribution between the two populations (p=0.02 OR= 0.614, 95% CI= [0.396-0.951]). By classifying the diabetic population according to genotypes: AA+AG vs GG, a significant difference was noted in fasting glucose,and calcaemia (p=0.012 and p=0.03). The rs972283 polymorphism can be involved in carbohydrate metabolism in diabetic patients.

CONCLUSIONS

T2DM is a complex and multifactorial disease. The predisposition factors are multiple, interacting with each other but also with the various environmental factors resulting in a great variability of the phenotype and personalized therapeutic responses. The elucidation of these different actors and the identification of new genes using molecular screening tools should lead to personalized medicine for each patient.