M001
Advanced Glycation End Products (AGEs) in diabetes mellitus and chronic disease

PERFORMANCE OF CKD-EPI PAKISTAN EQUATION, CKD-EPI, MDRD AND CG IN PREDICTING ESTIMATED GLOMERULAR FILTRATION RATE IN PAKISTANIS: COMPARISON WITH MEASURED CREATININE CLEARANCE

A. Sibtain 1, A.H. Khan 1, L. Jafri 1
1The Aga Khan University Karachi

BACKGROUND-AIM
National Kidney Disease Education Program strongly suggests reporting estimated glomerular filtration rate (eGFR) when serum creatinine (Cr) is measured and reported by laboratories. The aim of this study was to evaluate the results of 24 hour urinary creatinine clearance (CrCl) with eGFR using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), CKD-EPI Pakistan (CKD-EPI Pak), Cockcroft Gault (CG) and 4-variable Modification of Diet in Renal Disease (MDRD) equations.

METHODS
Laboratory data of subjects ≥ 18 years ordering 24 hour urinary CrCl from June to October 2015 was retrieved. Statistical comparison of eGFR using CKD-EPI, CKD-EPI Pak, CG and MDRD with the timed urine collection CrCl was done using regression analysis.

RESULTS
Mean age of the group (n=670) was 51.3 ±15.4 years, 55.7 % being males. Mean BMI of males and females was 27.8 ± 13 kg/m2 and 27.6 ±5.8 kg/m2 respectively. Mean GFR using 24 hour creatinine clearance was 57.1 ± 35.9 ml/min/1.73m2. Urinary creatinine clearance showed strong correlation with CG, MDRD, CKD-EPI and CKD-EPI Pak r=0.7, r=0.7, r= 0.82, and r= 0.83 respectively. Sensitivity was highest for the CKD-EPI Pakistan (84.7%). Similarly CKD-EPI Pakistan equation showed the highest agreement (88.7%) with CrCl compared to the other formulae.

CONCLUSIONS
The CKD-EPI Pak equation is more accurate and precise than the CG, CKD-EPI and MDRD in estimating GFR in Pakistani population
Advanced Glycation End Products (AGEs) in diabetes mellitus and chronic disease

CREATININE AND CYSTATIN C BASED EVALUATION OF RENAL FUNCTION AMONG OBESE SUBJECTS IN BENIN CITY, NIGERIA

A. Alaje 1, T. Adedeji 1, S. Idogun 2

1Department of Chemical Pathology, College of Health Sciences, Obafemi Awolowo University, Ile-Ife
2Department of Chemical Pathology, College of Medicine, UNIBEN, Benin City

BACKGROUND-AIM

INTRODUCTION: Obesity is a recognized worldwide epidemic having a high prevalence in developed countries and with increasing incidence documented in developing nations of the world. Several studies have shown that obesity is an independent risk factor for the development of chronic kidney disease apart from its link with diabetes mellitus, hypertension. This study evaluated the renal status of obese patients using both the established (creatinine) and new (cystatin C) markers of renal function.

METHODS

This was a cross-sectional study. A total number of fifty-nine (59) consenting adults attending the centre for disease control (CDC) of University of Benin Teaching Hospital (UBTH) for routine medical checks were recruited for this study using a simple random sampling. They were divided into obese and normal subjects based on their body mass index (BMI) and blood specimens collected with serum extraction after centrifugation for creatinine and cystatin C assays. Estimated glomerular filtration rate (eGFR) was calculated for each analyte using eGFR equations by CKDEPI. The eGFR results generated were compared in assessing renal function.

RESULTS

The obese subjects and the controls were age-matched (50.6±9.7 vs 50.7±7.8, p=0.2). The obese subjects had a significantly higher serum cystatin C and significantly lower eGFR-Cyst C than the controls (1.3 ±0.7 vs 0.9 ±0.4mg/L, p <0.001), and (75.4±38.9 vs 90.9± 25.1mL/min/m2, p< 0.001) respectively. There was a significant difference between the eGFR-Cr and eGFR-Cyst C mong the obese subjects (97.4±21.4 vs 75.4±38.9mL/min/1.73m2), p< 0.019).

CONCLUSIONS

Mild renal impairment exists among obese subjects; they should therefore be monitored to pre-empt further deterioration in renal function. There should also be a continuous campaign towards weight reduction. Cystatin C appears to be a better marker of renal function in obesity than serum creatinine.

KEY WORDS: OBESITY, CREATININE, CYSTATIN C
Advanced Glycation End Products (AGEs) in diabetes mellitus and chronic disease

**CYSTATIN C BASED EVALUATION OF RENAL FUNCTION AMONG OBESE PATIENTS IN BENIN CITY, NIGERIA**

A. Alaje, O. Adewolu, T. Adedeji

1Department of chemical Pathology, College of Health Sciences, Obafemi Awolowo University, Ile-Ife
2Department of Chemical Pathology, College of Medicine, UNIBEN, Benin City

**BACKGROUND-AIM**

Chronic kidney disease (CKD) is a recognized world public health problem in its epidemiology with burden of disease distributed globally. There is an annual growth rate of 8% for CKD probably due to the lack of consistent surrogate markers of kidney function to identify early stages of the disease. This study evaluated renal function of obese diabetic and obese non-diabetic patients using cystatin C and comparing it with creatinine.

**METHODS**

This was a cross-sectional study. A total number of eighty (80) consenting adults attending the centre for disease control (CDC) of University of Benin Teaching Hospital (UBTH) for routine medical checks and follow-up were recruited for this study using a simple random sampling. They were divided into three groups: obese euglycemic normotensives, obese diabetic normotensives and controls. Blood specimens were collected and separated after centrifugation for creatinine and cystatin C assays. Estimated glomerular filtration rate (eGFR) was calculated using equations by CKDEPI for creatinine and cystatin C. Results generated were compared in assessing renal function.

**RESULTS**

The obese subjects and the controls were age (52.0±9.4 vs 52.7±7.8, p=0.32) and sex matched. The obese subjects had a significantly higher serum Cystatin C and creatinine levels than the controls (1.7 + 1.0mg/L vs 0.9 + 0.4mg/L, p<0.001 and 106 + 38.5 µmol/L vs 91.4+ 24.4 µmol/L, p= 0.02 respectively). The obese subjects also had a significantly lower eGFR-Cystatin C (62.6 + 40.1mg/L) when compared with the controls (90.9+25mg/L), p<0.001; which were more pronounced in the obese diabetics than in the obese euglycaemic subjects. There was a significant difference between the estimated GFR based on creatinine and cystatin C among the subjects (88.1+ 24.3 and 62.6 + 40.0 ml/min/1.73m2, p<0.001).

**CONCLUSIONS**

Mild renal impairment exists in obesity which is made worse by an accompanying comorbidity such has diabetes mellitus. Insulin resistance syndrome is strongly related to the development of renal insufficiency among obese subjects. Serum Cystatin C appears to be a more sensitive marker of renal function in asymptomatic patients at risk of developing chronic renal disease.

**KEY WORDS: OBESITY, DIABETES, CYSTATIN C**
M004

Advanced Glycation End Products (AGEs) in diabetes mellitus and chronic disease

OXIDATIVE STRESS AND DIABETIC DEGENERATIVE COMPLICATIONS

Y. Chaabouni 2, M. Ouertani 2, W. Dabbebi 3, K. Mahdouani 1

1Laboratoire d’analyse, de traitement et de valorisation des polluants de l’environnement et des produits faculté de pharmacie Monastir
2Service des laboratoires hopital Ibn El Jazzar Kairouan
3Service des médecines hopital Ibn El Jazzar Kairouan

BACKGROUND-AIM

The diabetes is major problem on public health. Degenerative complications still represent the main cause of high morbidity and mortality. The generation of free radicals (oxidative stress) involve in the physiopathology of diabetic complications. This hypothesis is supported by evidence that many biochemical pathways strictly associated with hyperglycemia can increase the production of free radicals. The study evaluated activity of the key antioxidant enzymes superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPX) as well as total antioxidant status (TAS) in diabetic patients with degenerative complications, and investigated the correlation between parameters of glycemic control and parameters of antioxidant.

METHODS

The study included 106 diabetics, 43 of them patients with complications, with prevalence of 24% for cardiovascular problems, 23% for retinopathy, followed by neuropathy with 11% and finally 7% for nephropathy. All study subjects were examined and standardized according to essential parameters including laboratory assessment of blood fasting glycemic and glycated hemoglobin A1C (HbA 1C) concentration. The key antioxidant enzyme activity and TAS were assessed in blood samples using colorimetric Randox Kits

RESULTS

Diabetic patients with complications had significantly lower activities of SOD and GPX (p=0.017 and 0.019 respectively). There was no significant correlation of blood fasting glycemic and HbA1c concentration with either key enzyme activities or TAS.

CONCLUSIONS

The study demonstrated the presence of oxidative stress in diabetic patients with complications, which was not significantly influenced by glycemic control.
Advanced Glycation End Products (AGEs) in diabetes mellitus and chronic disease

LEAD ENVIRONMENTAL POLLUTION

N. Chaouali 1, A. Nouioui 1, M. Aouard 1, D. Amira 1, A. Hedhili 1
1Toxicology Department, Center of Medical Assistance and Emergency, 10 rue Aboul Kacem Chabbi, 1008 Montfleury, Tunis, Tunisia

BACKGROUND-AIM

Industrial, agricultural and urban development is inevitably accompanied by problems of environmental pollution. Industrial companies are releasing considerable quantities of heavy metal or metallic trace elements into the environment, mainly lead. The present work aims to identify the levels and sources of lead contamination in an industrial city in the northern region of Tunis-Tunisia.

METHODS

Oleander (Nerium oleander) leaves have been used to assess the degree of the lead environmental contamination. Samples were collected near the roads and industries located in the industrial city. Lead levels were determined by employing a Shimadzu AA-680 atomic absorption spectrophotometer equipped with a deuterium lamp and a transversely heated graphite tube atomizer (GFA-4B), an autosampler and recorder model ASC-6100.

RESULTS

Total concentrations of lead in contaminated oleander leaves were ranged from 8 to 137.8 µg/g (of dry matter), with a mean of 22 ± 29.71 µg/g. These levels are statistically significant relative to levels found in the control samples (0.91 ± 0.23 µg/g). The contamination levels of lead in samples collected near the roads was over the phytotoxicity threshold of 30 ppm. In samples collected around the electric accumulators factory, lead levels exceeded the threshold of 100 µg/g (fixed by the French national organization for standardization).

CONCLUSIONS

The data obtained in this study indicated that the study area has been affected by anthropogenic activity, in particular the electric accumulators industry, leading to a high accumulation of heavy metals compared with the natural background levels. Lead concentrations in Oleander leaves can be used as a powerful geochemical tracers of monitoring the impact of anthropogenic activities that seem to be the responsible source of pollution for metals in urban cities.
Advanced Glycation End Products (AGEs) in diabetes mellitus and chronic disease

DETECTION OF HEMOGLOBINOPATHIES (HBS AND HBC) FROM THE IDENTIFICATION OF HEMOGLOBIN VARIANTS IN GLYCOSYLATED HEMOGLOBIN ANALYZER

Y. Gamarra Morales 1, P. Gonzales Navarro 1, A. Alaoui 1, J.L. Garcia De Veas Silva 1, C. Garcia Rabaneda 1, C. Miralles Adell 1, T. De Haro Muñoz 1
1Hospital Complex of Granada

BACKGROUND-AIM
The aim of our study is to evaluate the benefits of screening for hemoglobinopathies based on the identification of abnormal hemoglobin by glycosylated hemoglobin analyzer.

METHODS
From August 2015 to January 2016, 20,000 outpatient EDTA-blood samples were included in this study. Glycosylated hemoglobin and hemoglobinopathies detection was performed by high performance liquid chromatography (HPLC) on the analyzer Menarini Diagnostics HA-8180V and HA-8160, respectively. Samples that displayed error or borderline results were submitted to a reference hospital laboratory for further evaluation using the analyzer Biorad Variant 2, which is able to separate fractions of HbS or Hbc. According to our protocol, all the samples that displayed any anomalous band on the glycosylated hemoglobin (HbA1c) chromatograms were further examined in other specific Hematology equipment for the detection of hemoglobinopathies.

RESULTS
Thirty-seven samples displaying abnormal bands by the glycosylated hemoglobin analyzer HA-8180V were randomly selected for further evaluation by the hemoglobinopathies analyzer HA-8160. Of the 37 selected samples, 11 (29.7%) were diagnosed as sickle cell trait and the remaining 26 (70.3%) as Hb S or C heterozygous hemoglobinopathies (ratio HbS / A approximately 35/65). These latter results were reported by the laboratory of Hematology with the following comment attached: “Additional test ordered by Hematology staff. Anomalous band was observed in the region S / C. Family screening for sickle cell trait is advised. Provided that the phenotype of sickle cell anemia can result from multiple combinations of molecular defects (Hb S / S, Hb S / C, Hb S / beta-logging), further confirmation by other tests (electrophoresis Hbs) is recommended.”

CONCLUSIONS
Taking advantage of the extra data provided by glycosylated hemoglobin analyzer (at no extra charge) we have described an approach for sickle cell trait screening in the daily laboratory routine. The importance of reporting on the trait or other altered hemoglobin is the possibility of further genetic counseling. In addition, information about the occurrence of this disorder in our area is also provided.
Advanced Glycation End Products (AGEs) in diabetes mellitus and chronic disease

PROTOCOL REVIEW THALASSEMIA SCREENING IN CENTRAL REFERENCE LABORATORY

Y. Gamarra Morales 1, P. Gonzales Navarro 1, J.L. Garcia De Veas Silva 1, A. Alaoui 1, M.L. Bellido Díaz 1, J.M. Villa Suarez 1, T. De Haro Muñoz 1
1Hospital Complex of Granada

BACKGROUND-AIM
The aim of our study is to review the protocols applied and the rejected test in our laboratory for the study of a possible hemoglobinopathy based on the results of the cell blood count (CBC) and the iron metabolism in patient attending primary care and specialized consults

METHODS
We reviewed the results of hemoglobin A2 (HbA2) and fetal hemoglobin (Hb F) during a period of six months. With these results, we determined the percentages of rejected and additional tests added by the Laboratory of Hematology and the percentage of normal and pathological results. Based on the results of hemoglobin, hematocrit, mean corpuscular volume, presence of polycythemia, red cell count in blood smear and iron metabolism (in absence of iron deficiency), the study of hemoglobin HbA2 and Hb F is added or rejected. The CBC was studied on an Advia analyzer (Siemens) and the hemoglobin fractions were studied on a HA-8160 analyzer (A. Menarini Diagnostic)

RESULTS
781 samples were received for the study of hemoglobinopathies. The laboratory rejected 365 samples (46.7%) with completely normal CBC. Of the 416 samples analyzed; 309 (74.3%) were compatible with normality and the remaining 107 (65 samples (61%) were added by the laboratory) the results were: 57 samples were beta thalassemia minor (13.7%), 10 samples were beta-delta thalassemia (2.4%) and 37 samples were structural hemoglobinopathies (Hb S and C) (8.9%). A beta thalassemia mayor, a Fanconi anemia diagnosed and a Diamond Blackfan anemia were detected. Of the 67% "thalassemia trait" found, 43 were added by the laboratory (64%)

CONCLUSIONS
About half the request for the study of hemoglobinopathies were rejected due to a normal CBC, avoiding unnecessary determinations and increasing efficiency in the management of our laboratory. About 10% of the analyzed samples, "thalassemia trait" were identified using the same sample after evaluation of the previous CBC and the iron metabolism by the hematologist and later addiction of the unsolicited hemoglobinopathies studies. This enabled us to rule out other causes of anemia or hemoglobinopathy identify an unsuspected avoiding hospital specialist consultation to generate a report as a guideline for the petitioner doctor, patient and / or family
Advanced Glycation End Products (AGEs) in diabetes mellitus and chronic disease

HEPATIC STEATOSIS AND DIABETES MELLITUS: RISK FACTORS, PATHOPHYSIOLOGY AND WITH ITS CLINICAL IMPLICATIONS

S.K. Dwedi 1, A. Mittal 1, B. Sathian 1, N. Chandrasekharan 1, A. Lekhi 1, R. Rahib 1
1Manipal College of Medical Sciences

BACKGROUND-AIM

The perception of nonalcoholic fatty liver disease (NAFLD) as an infrequent and benign condition is swiftly altering in developing countries as there has been an upsurge in non alcoholic fatty liver disease in Asia-Pacific region. NAFLD develops across all age groups and societies and is recognized to occur in 14%–30% of the common population. The foremost risk factors for NAFLD such as central obesity, diabetes mellitus, insulin resistance, dyslipidemia, hypertension, hypertriglyceridemia are currently predominant and puts a very large population at risk of evolving hepatic steatosis in the coming decades.

METHODS

It was a hospital based case control study carried out in the Department of Biochemistry of Manipal Teaching Hospital, Pokhara, Nepal. The variables collected were age, gender, fasting blood glucose, total cholesterol, low density lipoproteins, triglycerides, high density lipoproteins, very low density lipoproteins, aspartate transaminase, alanine transaminase.

RESULTS

Of the 200 patients of non alcoholic fatty liver disease patients with diabetes mellitus, all the variables except triglycerides shows insignificant disparity in relation to gender. The perceptible difference was observed in mean values of triglycerides for cases of NALFD between diabetes (218.25 ± SD 73.68) and non diabetic subjects (177.54 ± SD73.45) (p=.0001). The mean values of HDL did not illustrate much difference in cases of NALFD with diabetes (41.54 ± SD2.13) and non diabetic subjects (44.24 ± SD2.05).

CONCLUSIONS

Public health initiatives are undoubtedly of the essence to halt or turn around the global ‘diabesity’ pandemic, the causal basis of NAFLD. Management of patients with NAFLD should be aimed at treating metabolic risk factors such as hyperglycemia and hypertriglyceridemia. Successful lifestyle adaptation with increased exercise and decreased food intake is able to remove the accumulation of liver fat and can reverse insulin resistance.
M010
Advanced Glycation End Products (AGEs) in diabetes mellitus and chronic disease

EFFECT OF PHOTOBIOMODULATION (PBM) ON THE EXPRESSION OF EPIDERMAL GROWTH FACTOR (EGF) AND ACTIVATION OF ITS RECEPTOR (EGFR) IN DIABETIC WOUNDED CELLS

S. Jere 1
1University of Johannesburg

BACKGROUND-AIM

EGF is a mitogen protein that after binding to EGFR, play a critical role in wound repair by activating epidermal and dermal regeneration. EGF stimulate proliferation, cell growth and differentiation. These basic events are critically required in wound healing. EGF stimulates the proliferation of epithelial cells and in injury activates epidermal repair. In addition to this, EGF activates division and migration of epidermal and stromal cells including stimulating the development of blood vessels, and it also has mitogenic effects on keratinocytes. Furthermore, EGF stimulates fibroblast cell migration and transformation into fibrocytes, which are involved in wound contraction. Moreover, fibrocytes are involved in producing connective tissue proteins including collagens I, III and vimentin that play a vital role in wound repair. In vivo, EGF is synthesized by a variety of cells including Fibroblasts. However, due to the presence of increased proteases, diabetic chronic wounds have demonstrated de-regulated and reduced growth factor and growth factor receptors. In this case, the preconditioning of the chronic wound bed may be required for the bio availability of local growth factors which would stimulate corresponding receptors to initiate downstream events required for wound repair. Photobiomodulation (PBM) is a technique of using Low Intensity Laser Irradiation (LILI) or light emitting diodes (LED) to initiate therapeutic cellular effects presently used for reduction of pain or inflammation, modulation of the immune system, promoting wound repair process including regeneration of tissue. PBM effects the modulation of cytokines and accelerates angiogenesis in wound repair. The aim of this study was to analyse the effects of PBM on cell proliferation and migration through the activation of EGFR by the Janus Kinase 2 (JAK 2) in diabetic wound healing.

METHODS

We used commercially purchased human skin fibroblast cells (ATCC, WS1) to determine the effect of PBM on the activation EGFR by JAK2. In this investigation, cells were exposed to a wavelength of 660 nm and energy density of 5 J/cm2 with a power output of 100 mW. We continuously grew the cells in complete media with an additional 17 mM/L D-glucose to achieve a diabetic wounded model and a monolayer of cultured cells in 35 mm diameter culture dishes was wounded by performing a central scratch using a sterile 1 mL pipette. Cultured cells in the presence or absence of exogenous EGF (rhEGF) were irradiated and incubated for 48 h. Non-irradiated cells served as control. Cellular migration was monitored microscopically every 24 h for 48 h using an inverted light microscope. Cell proliferation was assessed by incorporation of 5-bromo-2-deoxyuridine (BrdU). Viability was assessed by ATP and trypan blue exclusion assays. Enzyme linked immunosorbent assay (ELISA) and immunofluorescence microscopy were used to analyse the phosphorylation of EGFR and JAK2. AZD1480, a JAK2 inhibitor was used to inhibit cell proliferation, migration and wound repair.

RESULTS

The results indicate that PBM activates cellular proliferation and migration by activation of EGFR by JAK2 in diabetic wounded fibroblast cells.

CONCLUSIONS

Combined therapeutic agents targeting different pathways and use of PBM may increase the response rate of diabetic wound healing. More research into understanding the molecular mechanisms and biological changes in the cell, specifically the cytokine and growth factor pathways after PBM is warranted.
M011
Advanced Glycation End Products (AGEs) in diabetes mellitus and chronic disease

THE EVALUATION OF PROTEIN OXIDATION IN THE RATS WHICH INDUCED BY STREPTOZOTOCIN

D. Meltem 2, K. Kader 1, Y. Cevat 1
1Erciyes University Medical Faculty Biochemistry
2Kemerburgaz University Medical Faculty. Biochemistry

BACKGROUND-AIM
Aim: Diabetes mellitus (DM) is a chronic disorder and characterized by the development of long-term complications. Beyond toxic effects of hyperglycemia to tissues, several molecular mechanisms including genetic modulation are defined. Various complex mechanisms like elevated level of carbonyl stress, oxidative stress and advanced glycation end products (AGE) are thought to be responsible for the development of diabetic complications. Methylglyoxal (MGO), a precursor of advanced glycation end products (AGE), is detoxified in the organism by Glyoxalase through Glyoxalase I (GLO I) and GLO II. How this system is affected under diabetic conditions is still being studied.

METHODS
The most common animal model of human diabetes is STZ-induced diabetes in the rat. Four study groups, each containing ten Sprague Dawley rats, were defined as control, MEL, STZ and STZ-MEL. STZ and STZ-MEL groups were given a single 50 mg/kg dose of STZ to induce diabetes. MEL, 25 mg/kg was given intraperitoneally to MEL and STZ-MEL groups on a daily basis for 42 days. During the study, the rats were weighed weekly. Blood and 24 h urine samples were collected at the beginning and at the end of study, and also once in two weeks and weekly, respectively. Glucose and Hb A1c were measured in the blood samples.

At the end of study, the levels of MGO, one of the AGE precursors, and the activities of GLO I and GLO II enzymes, members of glyoxalase detoxification system were also determined in only tissue samples.

RESULTS
Blood and urine glucose levels were found to be high in rats. Rats which were excreting gradually more urine lost weight during the study. Although STZ group had been shown to have higher tissue MGO levels and lower GLO I and GLO II activities, MEL treatment had suppressed high levels of MGO and increased enzymatic activities in STZ-MEL group.

CONCLUSIONS
In this study, we have shown that reducing MGO tissue levels in chronic diabetes to almost normal level and that the GLO system suppressed in diabetic rats are preserved with MEL, in other words, GLO I and GLO II activities increased. It has been shown that STZ-induced diabetic rats had high MGO levels and the suppression of GLO detoxification system indicates that AGE formation in diabetes is inevitable. Therefore, the usage of antioxidants such as MEL may be suggested to prevent diabetic complications, at least partly in diabetic patients.
Advanced Glycation End Products (AGEs) in diabetes mellitus and chronic disease

EFFICIENCY MONITORING OF THERAPY BY FRUCTOSAMINE AS BIOMARKER IN YOUNG AND DM PATIENTS

D. Pap 2, V. Canic 1
1Municipal Institute for Lung diseases and Tuberculosis, Belgrade, Serbia.
2Students Health Protection Institute, Novi Sad, Serbia

BACKGROUND-AIM

The measurement of fructosamine (FRU) is useful in monitoring short to medium glycemic control in DM, over the past 2-3 weeks. Diabetes mellitus (DM) can be assessed by the long term monitoring and control of glucose levels as short term indicator. When blood glucose levels are abnormally elevated the concentration of fructosamine also increases. The aim of this study was to evaluate diagnostic efficiency for monitoring of DM by fructosamine assay.

METHODS

The studied subjects were the control group (136 healthy students) and the experimental group (188 DM patients). The experimental group divided in four groups: M1 – 54 non-insulin dependent DM patients (NIDDM) on diet; M2 - 68 NIDDM patients on oral antidiabetes therapy; M3 –32 NIDDM patients on insulin; M4 –34 insulin dependent patients (IDDM). Patients were both sexes, age matched and monitoring in last 3 weeks. We performed FRU determinations (by NBT colorimetric method). Glucose concentration was measured by GOD-PAP method.

RESULTS

FRU and glucose values in serum were significantly higher (p< 0,01) in all groups of patients compared to the control group of young during whole period of monitoring of DM. FRU was significantly correlated with glycemia over the past 2 weeks. The results of examined parameters in all groups have shown the following values: M1 for glucose 7,36±1,39; and FRU values ranged from 258-320 μmol/l; M2 9,60± 3,77; FRU 346-386 μmol/l; M3 12,25±3,62; FRU 447-509 μmol/l; M4 15,01 ± 5,95; FRU 497-587 μmol/l; control group 5,05 ±0,75; FRU 174-225 μmol/l.

CONCLUSIONS

Simultaneous determination of both parameters allows us to emphasize the recent metabolic decompensation. The results suggest that fructosamine assay is useful medium-term marker to monitor diabetic patients in regard to their therapy.
Advanced Glycation End Products (AGEs) in diabetes mellitus and chronic disease

A SIMPLE MEANS TO DIFFERENTIATE LABILE HBA1C FROM HAEMOGLOBIN VARIANT ON BIO-RAD VARIANT II TURBO CATION-EXCHANGE HPLC METHOD

W.L. Cheng 1, S. Sethi 2, T.P. Loh 2, B. Pratumvinit 1
1Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital, Bangkok
2Department of Laboratory Medicine, National University Hospital, Singapore

BACKGROUND-AIM

Accurate measurement of glycated haemoglobin A1c (HbA1c) is important for optimal management of diabetes. Cation-exchange high performance liquid chromatography (CA-HPLC) methods can be interfered by Hb variants, which are prevalent in many parts of the world, including South-East Asia (prevalence: Singapore: 3.5%; Thailand: up to 30%). The Bio-rad Variant II Turbo is a CA-HPLC method. Certain Hb variants (e.g. HbJ) can elute in the labile HbA1c window that precedes HbA1c, and interfere with its measurement. Labile HbA1c is a reversible intermediary formed between glucose and Hb acutely after ingestion of carbohydrate. It is important to differentiate labile HbA1c formed after a meal from Hb variant.

METHODS

We derived the reference interval for labile HbA1c from subjects without Hb variant by mixing EDTA whole blood sample and normal saline at 1:19 ratio and incubating it in a water bath at 37°C for 4 hours before re-analysis. The reference intervals were then applied on samples with known Hb variant after identical incubation condition.

RESULTS

The reference intervals for subjects with normal Hb (n = 121) were expressed as labile HbA1c:HbA1c ratio, and they were 0.18-0.55 and 0.13-0.41 before and after 4 hours of incubation, respectively. By contrast, the minimum and maximum labile HbA1c:HbA1c ratios for the different Hb variants were:

- HbE (n = 12), pre-incubation: 0.12-0.37, post-incubation: 0.11-0.25;
- HbS (n=10), pre-incubation: 0.20-0.27, post-incubation: 0.14-0.21;
- HbD (n=13), pre-incubation: 0.17-0.27, post-incubation: 0.12-0.21;
- HbJ (n=10), pre-incubation: 0.74-1.03 post-incubation: 0.76-1.02.

CONCLUSIONS

Incubating EDTA whole blood samples in normal saline at 37°C for 4 hours reduces the labile HbA1c fraction. The use of the labile HbA1c:HbA1c ratio can effectively differentiate Hb variants eluting in the labile HbA1c window, particularly when it does not decrease after incubation, as shown by subjects with HbJ. Laboratory practitioners can use this simple manipulation to clarify suspicious peak eluting at labile HbA1c window.
Advanced Glycation End Products (AGEs) in diabetes mellitus and chronic disease

EVALUATION OF A POINT OF CARE INSTRUMENT FOR GLYCATED HAEMOGLOBIN (HbA1c) IN A COMMUNITY AT RISK OF DEVELOPING DIABETES

M. Rensburg 1, A. Zemlin 1, J. Esser 1, T. Matsha 2, A. Kengne 4, R. Erasmus 3
1Division of Chemical Pathology, Department of Pathology, Faculty of Medicine and Health Sciences, National Health Laboratory Service (NHLS) and Stellenbosch University, Cape Town, South Africa
2Department of Biomedical sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa
3Division of Chemical Pathology, Department of Pathology, Faculty of Medicine and Health Sciences, National Health Laboratory Service (NHLS) and Stellenbosch University, Cape Town, South Africa
4Non-Communicable Diseases Research Unit, South African Medical Research Council, Cape Town, South Africa.

BACKGROUND-AIM
Point of care testing (POCT) has the potential to improve healthcare by improving turnaround time, enabling quick clinical decision making and causing less inconvenience for the patient. However, test performance such as accuracy and reliability needs to be ensured. These tests are often performed outside the laboratory yet strict quality needs to be maintained. HbA1c has recently been recommended to screen for diabetes and even prediabetes. Previous studies have raised issues that may prevent the use of HbA1c POCT for this purpose. The Western Cape in South Africa has previously been found to have a high prevalence of diabetes and POCT for early diagnosis would be beneficial and reduce morbidity. The IDF guidelines state that the use of HbA1c for the diagnosis of diabetes and prediabetes requires stringent quality assurance and standardized assays. The use of POCT HbA1c for this purpose has not yet been studied in this population. The aim of this study was to determine the correlation between HbA1c measured on capillary blood on the ALERE Afinion™ POCT instrument and laboratory HbA1c determined by Biorad D10 – HPLC, an accredited method (NGSP Level 1 certified). A secondary aim was to evaluate the performance of HbA1c determination on the Afinion™ POCT instrument in capillary and venous samples.

METHODS
Participants from the Bellville South study were recruited and had their HbA1c value determined by Afinion™ POCT and Biorad HPLC method. Pearson’s correlation test was used to assess the association between the two methods and systematic bias was examined using Bland-Altman plots. A total allowable error of 3% was used to determine statistically significant differences between the laboratory and POCT measurements. Results obtained on the Afinion™ HbA1c POCT were additionally compared using capillary and venous samples in a subset of 55 participants.

RESULTS
A total of 722 participants with a mean age of 51.5 years were recruited. The mean HbA1c as determined using the laboratory HPLC method was 6.4% (46.3 mmol/mol) versus 6.3% (45.0 mmol/mol) using the POCT method (r=0.974). The mean percentage difference was within the 3% total allowable error. Good correlation was obtained between the POCT venous versus capillary measurements [r=0.995; mean HbA1c value on both 6.2% (44.0 mmol/mol)].

CONCLUSIONS
HbA1c as determined by POCT on the Afinion™ correlated well with the laboratory HPLC determination. In our resource-limited setting with a high prevalence of diabetes, POCT determination of HbA1c would lead to more rapid diagnosis and treatment of diabetes and decreased morbidity. This may be a potential cost-saving initiative.
Advanced Glycation End Products (AGEs) in diabetes mellitus and chronic disease

HBA1C METHOD PERFORMANCE AND UTILIZATION AT PUBLIC HEALTH LABORATORIES IN SOUTH AFRICA-A SIGMA METRIC EVALUATION

A. Zemlin ¹, M. Rensburg ¹, J. Esser ¹, R. Erasmus ¹

¹Division of Chemical Pathology, Department of Pathology, Faculty of Medicine and Health Sciences, National Health Laboratory Service (NHLS) and Stellenbosch University, Cape Town, South Africa

BACKGROUND-AIM

Performance goals for HbA1c assays are required to ensure accurate and reliable results for clinical use. A sigma metric evaluation that combines accuracy and precision through the concept of a total allowable error has been shown to provide a simple integrated method performance indicator. Little is known about HbA1c analytical performance at public health laboratories in South Africa. An HbA1c analytical performance audit was performed using a sigma metric approach. The purpose of this study was to audit various public health laboratories to identify the type of assay used to determine HbA1c and to evaluate their analytical performance using a sigma metric approach.

METHODS

A retrospective audit of HbA1c external quality control data was conducted. Data was obtained by distributing a questionnaire and requesting end-of-cycle external quality control and internal quality control reports. Linear regression analysis was performed on the data to determine the standard deviation and bias at the medical decision limit of 6.5% HbA1c. The sigma metric was calculated using a total allowable error of 0.46% HbA1c and 2-sigma as the minimum performance indicator. Results are plotted on a sigma proficiency assessment chart.

RESULTS

All the laboratories subscribed to HbA1c external quality control programmes using samples of unknown commutability. All the methods were NGSP certified. Only 2 from the 9 instruments evaluated had a sigma-metric above 2. The Tosoh 8 ion-exchange HPLC platform showed the best performance overall.

CONCLUSIONS

The majority of HbA1c methods evaluated did not achieve the 2 sigma performance goal. The sigma metric evaluation is a practical method to evaluate HbA1c performance in our setting. However, it is important to recognise the limitations of this method when using routine external quality control data.
M016
Advanced Glycation End Products (AGEs) in diabetes mellitus and chronic disease

ETHANOLIC EXTRACTS OF NAUCLEA LATIFOLIA, MANIHOT ESCULENTA LEAF AND LEAF STALK NORMALIZED EARLY SYMPTOMS OF TYPE 1 DIABETES IN ALLOXAN-INDUCED DIABETIC RATS

E.I.O. Ajayi 1, M. Okiemen 1, C.C. Uche 1, A.D. Adewale 1
1Biochemistry Department, Osun State University, Osogbo, Nigeria

BACKGROUND-AIM
Nauclea latifolia Sm is an evergreen multi-stem shrub, whose leaves have been claimed to be traditionally useful in the treatment of various ailments. Manihot esculenta Crantz leaves and stalk are also known to be a rich source of useful nutritive and medicinal compounds. Therefore we sought to ascertain the possibility that the extract will exhibit lipid-lowering and antidiabetic activities.

METHODS
Crude extracts were prepared from Nauclea latifolia leaves, Manihot esculenta leaves and leaf stalks by cold extraction in 97% ethanol after percolation for 24 hours. The crude extracts were tested for their antidiabetic abilities in vivo using high energy diet-manipulated, alloxan-induced diabetic rats.

RESULTS
Oral administration of the plant extracts at 200mg/kg body weight over 30 days exhibited pronounced antidiabetic effects as shown by their ability to reverse hyperglycaemia and hyperlipidemia in the diabetic rats. Fasting plasma glucose (FPG) levels were significantly reduced by Nauclea latifolia and Manihot esculenta leaf extracts (63.79% and 51.41%, respectively) compared to the standard antidiabetic drug, glibenclamide (54.44% and the vehicle, olive oil (8.82%); while Manihot esculenta leaf stalk extract had no significant effect on FPG (23%). Decreased triglyceride and increased cholesterol levels were recorded for Nauclea latifolia in the serum (57.24 ± 1.23 mg/dL and 332.85 ± 7.15 mg/dL, respectively) and skeletal muscle (72.96 ± 3.19 mg/dL and 576.27 ± 8.47 mg/dL, respectively). Increased triglyceride and decreased total cholesterol levels were observed for Manihot esculenta leaf in the serum (109.45 ± 8.34 mg/dL and 270.76 ± 7.04 mg/dL, respectively) and skeletal muscle (125.80 ± 2.15 mg/dL and 68.22 ± 7.58 mg/dL, respectively). Decreased triglyceride levels were observe in the serum (57.98 ± 4.78 mg/dL) and skeletal muscle (66.83 ± 6.39 mg/dL), while cholesterol was also increased in the skeletal muscle (200.84 ± 2.52 mg/dL), but decreased in the serum (23.74 ± 1.94 mg/dL) for Manihot esculenta leaf stalk, compared to the control group (57.24 ± 1.23 mg/dL and 72.97 ± 3.20 mg/dL; 322.85 ± 7.16 mg/dL and 76.27 ± 4.75 mg/dL, respectively).

CONCLUSIONS
This shows that Nauclea latifolia and Manihot esculenta leaf extracts are more promising natural products compared to Manihot esculenta leaf stalk extract for management of pre-diabetes, having normalized early symptoms of type 1 diabetes.
Advanced Glycation End Products (AGEs) in diabetes mellitus and chronic disease

RELATIONSHIP BETWEEN ENDOGENOUS SECRETORY RECEPTOR FOR ADVANCED GLYcation END-PRODUCTS WITH CORONARY ARTERY CALCIFICATION SCORE IN MALE WITH TYPE 2 DIABETES MELLITUS

D.Y. Daulay 2, S. As’ad 1, A. Wijaya 1

1Hasanuddin University  
2Prodia Clinical Laboratory

BACKGROUND-AIM
The number of people with diabetes has been rising in many countries, including Indonesia. Diabetic patients may suffer a number of debilitating complications such as retinopathy, nephropathy, neuropathy, and atherosclerosis resulting in cardiovascular, cerebrovascular, or peripheral vascular disease. Many study have report that advanced glycation end-products (AGEs) and the receptor for AGEs (RAGE) system play an important role in the development of diabetic macrovascular complications. Endogenous secretory RAGE (esRAGE), has been identified as an alternatively spliced form of RAGE. esRAGE is secreted extracellularly and may act as decoys leading to the neutralization of the actions of AGE. However the correlation between serum esRAGE and vascular calcification in adult type 2 diabetes mellitus is unknown yet. This study aims to determine the relationship between esRAGE with progression of vascular calcification in male with type 2 diabetes mellitus.

METHODS
This study is an observational study with cross sectional design to adult type 2 diabetes male. A total of 61 type 2 diabetes adult male were included in this study, age range from 36 to 63 years old. Serum esRAGE concentration were quantified by ELISA principle. The progression of vascular calcification is determined by coronary artery calcification (CAC) score was measured by dual slice computed tomography (DSCT). All assays were performed according to manufacture instruction. Statistical analysis was perform with SPSS for windows ver 20. Significant value were define as alpha level < 0.05 based on two tailed-tests.

RESULTS
We categorized subjects into several groups based on the CAC score, 11 subjects were minimal coronary artery calcification (score 1-10), 18 subjects were mild coronary artery calcification (score 11-100), 25 subjects were moderate coronary artery calcification (score 101-400) and 7 subjects were extensive coronary artery calcification (score > 400). The results of this study shows there is no significant correlation between esRAGE and CAC score in total subjects, but in minimal coronary artery calcification group, there is negative significant correlation between esRAGE and CAC score ($r=-0.563$, $p=0.036$), whereas in the mild to extensive coronary artery calcification group, there is no significant correlation.

CONCLUSIONS
The results of this study indicate that in the type 2 diabetes, esRAGE plays a role in the early stages of calcification to protect the vascular from calcification through RAGE-mediated inflammatory inhibition. Further study is needed to validate that esRAGE may serve as a therapeutic target for intervention in the control of type 2 diabetes.
Advanced Glycation End Products (AGEs) in diabetes mellitus and chronic disease

INTERRELATIONSHIP BETWEEN TFE3 AND RAG GTPASE C/D DEPENDING ON NUTRITIONAL STATUS

M.Y. Kim 1, Y.H. Ahn 1

1Department of Biochemistry and Molecular Biology, Yonsei University College of Medicine, Seoul

BACKGROUND-AIM

Recently it was reported that MiTF/TFE family might function as critical factors in nutrient sensing and maintenance of cellular homeostasis. In amino acid sufficient state, active Rag GTPase binds the mTORC1 component raptor and recruits mTORC1 to lysosome. In addition, active Rag GTPase interacts with TFE3 and this interaction facilitates the mTORC1-dependent phosphorylation on TFE3. In starvation state, TFE3 is translocated to the nucleus and bound to the CLEAR elements present in the promoter region of varieties of lysosomal genes.

METHODS

To investigate the target gene of TFE3, micro assay was performed using Affymatrix chip with mouse liver exogenously overexpressing TFE3. ChIP assay and luciferase assay showed that TFE3 activates the RagC/D promoter activity. TFE3 mediated up-regulation of RagC/D was tested using RT-qPCR and western blot assay.

RESULTS

In silico search suggested that there are several CLEAR elements in the Rag GTPase C and D (RagC/D) promoter. In this study, we demonstrate that TFE3 is responsible for the up-regulation of RagC/D gene expression in mouse liver. ChIP assay showed that TFE3 binds directly to RagC/D gene promoter. Transduction of adeno-TFE3 to mouse liver increased RagC/D mRNA and protein level. Interestingly, Adenovirus mediated shTFE3 treatment down-regulated the gene expression of RagC/D Hepa1-6 cell.

CONCLUSIONS

From this study, it is concluded that TFE3 activates the gene expression of Rag GTPase C/D by direct binding on the CLEAR element of the promoter. Thus, TFE3 might act as direct transcription factor modulating RagC/D gene expression. Furthermore, it is expected that both TFE3 and Rag GTPase C/D molecules affect to each other through regulating the gene expression and cellular localization, respectively.