Abstract

Introduction:

Former studies have shown how preanalytical factors, specifically: repeatedly freezing and thawing of samples, as well as pre-storage centrifugation conditions, affects the stability of biomarkers. However, there is a lack of evidence about how these preanalytical factors affect the stability of diabetic associated biomarkers. Therefore, the aim of the present study was to investigate the effects of repeated freezing and thawing as well as pre-storage time of the analysis insulin, glucagon, Glucagon Like Peptide-1(GLP-1), Glucose-dependent Insulinotropic Polypeptide(GIP), leptin, c-peptide and Polypeptide YY(PYY), when analyzing the samples using new technology MESO Quickplex SQ 120 from Meso Scale Diagnostics LLC (MSD). This new technology is currently being used for research purposes at Steno Diabetes Center Copenhagen (SDCC), where this project was carried out.

Material and methods:

Plasma samples from a previous study were used to examine the long-term effects of repeated freeze/thaw cycles. Fresh blood samples from 10 healthy individuals were collected in BD™ P800 Blood Collection System. 3 blood samples from each test person were stored at room temperature (RT) for 0 h, 1 h and 3 h before centrifugation. Plasma from each sample was divided into 3 aliquots. An aliquot from each sample was analyzed immediately after, and the remaining aliquots were exposed to 3 and 5 repeated freeze/thaw cycles. A total of 120 aliqouts were measured. All samples were analyzed using the U-Plex Diabetes Combi 1 (hu) kit from MSD.

Results:

In this project we found that the stability of all 7 biomarkers were affected by pre-centrifugation storage time at RT and repeated freezing and thawing. C-peptide was the only biomarker in this study where there was a statistically significant change in concentration from 0 to 3 hours of storage at RT ($p = < 0,05$). GIP, GLP-1 and PYY stability was mostly affected by repeated freezing and thawing, with $p = < 0,05$ after almost every storage time and freeze/thaw cycles. Glucagon's stability was greatly affected by long term freezing and repeated freezing and thawing ($P = 0,02$). The glucagon concentration decreased with 36 % after long term freezing and 5 freeze/thaw cycles.

Conclusions:

The results demonstrated that the stability of insulin, c-peptide, glucagon, GIP, GLP-1, leptin and PYY is affected by pre-centrifugation storage time at RT and repeated freezing and thawing. These pre-analytical factors can cause a statistic significant change in the concentration of the biomarkers and impact the diagnosis, treatment and research related to diabetes.

Indstilling

Rebekka undersøger alt til bunds, så jeg lærer nye ting af at arbejde sammen med hende. Desuden tænker hun ud af boksen. F.eks. mangler hun et godt statistikprogram til at lave grafer og til projektet. Det finder hun da bare på internettet. Og finder selv ud af at anvende det! Hendes bachelorprojekt er ekstremt velskrevet og reflekterende. Og alle resultater perspektiveres direkte til klinisk betydning samt anvendelse. Ligeledes er hendes forsøg veludført samt gennemtænkt, på trods af, at forsøgene er meget omfattende. Jeg er sikker på at Rebekka har både modet samt evnerne til at medvirke til udvikling af fremtidens bioanalytiker profession. Projektet genererer ny viden, der kan gavne læger, bioanalytikere og sygeplejersker til at forstå hvordan præ-analytiske tilstande kan påvirke stabiliteten af biomarkører. Således kan usikkerheden på prøveresultaterne reduceres og kvaliteten af prøvesvar i klinikken og forskningen kan forbedres. Dette vil i sidste ende gavne diabetes patienter, så de kan få stillet den rette diagnose i tide og få den bedst mulige behandling. Resultaterne kan anvendes til at forbedre retningslinjerne for opbevaring af prøver i biobanker, da projektet viser at antallet af tø/frys cykler influerer på analyseresultaterne af flere af de inkluderede biomarkører.