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Short communications

How to perform pregnancy diabetes screening correctly

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Pregnancy diabetes (GDM) is associated with both higher rates of morbidity and mortality, and is diagnosed in thousands of women each year (1). Fasting glucose and glucose tolerance tests form the backbone of diagnosis. Since in most cases the diagnosis is based on a single laboratory assessment, any (pre)analytical error may result in a faulty diagnosis. Here, we shortly describe our recent studies in the context of optimization of pregnancy diabetes screening.

Currently, the global update of GDM guidelines based on the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study (2) has sparked fierce debate, as the protocol that needs to be followed to use these new cut-off values are cumbersome, expensive and hard to comply to (3). More specific, this protocol calls for a turn-around-time (TAT) of pre-analysis of less than 5 minutes, cooling of phlebotomy tubes in ice and immediate freezing or measurement of isolated plasma after centrifugation. Deviation from these procedures most likely results in underdiagnosis (3).

In vitro, glucose levels start to drop immediately after phlebotomy due to glycolysis. To minimize glycolysis, one should place the sample tube in an ice-water slurry, and plasma should be separated from the cells within 30 min. If that cannot be achieved, a tube containing a rapidly effective glycolysis inhibitor should be used for collecting the sample. Importantly, tubes with only enolase inhibitors (i.e. sodium fluoride) should not be relied on to prevent glycolysis as sodium-fluoride is far from capable to prevent glucose loss during the first hours after phlebotomy (4).

Method

We have performed a well-controlled study to assess the rate of glycolysis in commonly used phlebotomy materials (5). In short, blood was drawn from apparently healthy volunteers divided into 4 groups of 22 subjects each. 5 test tubes were drawn from each volunteer; a lithium-heparin control tube (BD Vacutainer, 367374) and 4 additional test tubes of 1 specific tube type. lithium-heparin, NaF-EDTA-citrate (TerumoVenosafe VF-052SFC), NaF-oxalate (BD Vacutainer 368921) and NaF-EDTA (BD Vacutainer 368521) tubes were tested. The control tube was placed in an ice/water slurry for a maximum of 15 minutes prior to centrifugation (5 minutes, 2800 g) as was 1 of the test tubes. The other test tubes were stored at room temperature, and centrifuged at set time points (30, 60 and 120 minutes).

Results

We found that only tubes containing NaF-EDTA-citrate were able to keep glucose levels stable to control levels, and thus to levels similar to tubes handled according to the recommended procedure. In addition, we found that glucose concentration was equally unstable in lithium-heparin, NaF-oxalate and NaF-EDTA tubes during the first two hours after phlebotomy confirming that addition of sodium-fluoride had no effect on glycolysis in this timeframe. On average, glucose concentration dropped 0.3 mM/h in all non NaF-EDTA-citrate tubes, which means about 5 to 10%, depending on the start glucose level (5).

As it is highly likely that laboratories deviate from the aforementioned elaborate HAPO procedure they should use phlebotomy material that contains direct glycolysis inhibitors, such as NaF-EDTA-citrate. In a nationwide survey, we have asked heads of clinical laboratories to report on the local pre-analytical phase of glucose measurements (6). As expected, we found that pre-analysis turnaround time was generally longer than 30 minutes, with most laboratories having a TAT of more than 60 minutes (n=14 of 25). To prevent glycolysis, most laboratories indeed relied on the addition of inhibitors. However, in contrast to the recommended procedures, only very few laboratories use direct glycolysis inhibitors (n=1 of 25), and most use tubes that contained only sodium-fluoride (n=22 of 25).

Medical decisions in all laboratories that participated in the study were not based on the recent HAPO study, but based on either the NIV (Nederlandse Internisten Vereniging, richtlijn “Diabetes en Zwangerschap”) or NVOG (Nederlandse Vereniging voor Obstetrie en Gynaecologie, richtlijn “Diabetes mellitus en zwangerschap”) guideline. It is important to realise that both guidelines are ultimately based on the 1999

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WHO recommendation for definition, diagnosis and classification of diabetes mellitus, in which the exact same elaborate protocol as was used in HAPO was used to define the cut-off values for pregnancy diabetes (7).

Combined, these data show that most laboratories do not adhere to the recommended (pre-)analytical procedures, but employ a protocol that results in glucose measurements that are ~ 0.3 mM too low.

As it is unreasonable to expect laboratories to switch to unpractical protocols in which test tubes are either immediately put in an ice/water slurry or centrifuged immediately, the use of NaF-EDTA-citrate tubes would be recommendable. To be sure that the use of NaF-EDTA-citrate tubes is compatible with the recommended procedures, we have performed a clinical validation [8]. Blood was drawn from pregnant women that were subjected to a 75 gram glucose tolerance test (GTT; n = 50) and collected in lithium-heparin (BD Vacutainer, 367374) and NaF-EDTA-citrate (Terumo Venosafe VF-052SFC) tubes. All lithium-heparin tubes were handled STAT, and centrifuged (4400 g, 5 minutes) directly after phlebotomy. All NaF-EDTA citrate tubes were centrifuged 60 minutes after phlebotomy, forming a feasible laboratory procedure. Plasma was isolated immediately after centrifugation, kept on ice until storage, and subsequently stored at -80° Celcius until further analysis.

Glucose concentrations determined by STAT laboratory analysis and determined using the feasible protocol agreed extremely well at both 0 and 120 minutes of GTT . No relative bias was found at t = 0 (best fit 1.01, 95% CI 0.91 to 1.10) or t = 120 (best fit 0.96, 95% CI 0.92 to 1.01). In addition, no significant offset (absolute bias) was found between methods at t = 0 (best fit – 0.03, 95% CI – 0.07 to 0.00) and only a very small statistically significant but clinically irrelevant bias was found at t = 120 (best fit 0.06 mM 95% CI 0.00 to 0.13). In conclusion, the use of citrated phlebotomy material with a pre-analytical TAT of 60 minutes enables a feasible GTT protocol, that yields results that are identical to WHO recommended STAT protocol based on heparinized plasma analysis.

Conclusion

Many Dutch clinical laboratories employ inadequate protocols in the context of GDM screening, mainly due to the infeasibility to adhere to the cumbersome gold standard. Since they do use the cut-off values determined using the gold standard, this results in underdiagnosis of GDM. Given the annual number of live births in The Netherlands each year (~175.000 in 2014) and the high incidence of pregnancy diabetes in the screened population (~10%) it is likely that hundreds or thousands of patients do not receive proper care due to incorrect laboratory diagnostics. A new protocol using a new phlebotomy tube type containing a NaF-EDTA-citrate additive results in laboratory and clinical diagnosis that are 100% identical to the gold standard, without the need to further adapt current procedure characteristics such as preanalytical TAT.

References

1. Arendz IJ, Oomen PHN, Wolthuis A, Velde NM van der, Kroese JA, Veen I van der, Veeger NJGM. Prevalentie van diabetes gravidarum bij risicozwangeren. Ned Tijdschr Geneeskd. 2013; 157: A5409.
2. Metzger BE, Lowe LP, Dyer AR, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med. 2008; 358: 1991-2002.
3. van den Berg SAA, van Thiel SW. Thelen M. Updating pregnancy diabetes guidelines: is (y)our laboratory ready? Clin Chem Lab Med. 2016 (published ahead of print)
4. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, Lernmark A, Metzger BE, Nathan DM. Executive summary: guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem. 2011; 57: 793-798.
5. van den Berg SAA, Thelen MHM, Salden LPW, van Thiel SW, Boonen KJM. It takes acid, rather than ice, to freeze glucose. Sci Rep. 2015; 5: 8875.
6. van den Berg S, Thelen M, Boonen K. Inventarisatie van de (pre)analytische aspecten van de glucose bepaling in nederlandse laboratoria; tijd voor uniformiteit. Ned Tijdschr Klin Chem Labgeneesk. 2015; 40: 68-71.
7. World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. 1999. Geneva.
8. van den Berg SAA, de Groot MJM, Salden LPW, Draad PJGJ, Dijkstra IM, Lunshof S, van Thiel SW, Boonen KJM, Thelen MHM. Pregnancy diabetes: A comparison of diagnostic protocols based on point-of-care, routine and optimized laboratory conditions. Sci Rep 2015; 5: 16302.