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Laboratory diagnosis of gestational diabetes: An *in silico* investigation into the effects of pre-analytical processing on the diagnostic sensitivity and specificity of the oral glucose tolerance test

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ABSTRACT

Introduction: Delayed separation of red cells from plasma causes pre analytical glucose loss, which in turn results in an under-diagnosis of GDM (gestational diabetes) based on the OGTT (oral glucose tolerance test). *In silico* investigations may help laboratory decision making, when exploring pragmatic improvements to sample processing.

Methods: Late pregnancy 0, 1 and 2 h 75 g OGTT values were obtained from two distinct populations of pregnant women: 1. Values derived from the HAPO (Hyperglycemia and Adverse Pregnancy Outcome) Study and 2. New Zealand women identified as at higher risk of GDM by their caregivers, undergoing OGTT during routine antenatal care. In both populations studied, *in silico* modelling focussed on the effects of pre-analytical delays in plasma separation, when using fluoride collection tubes.

Results: Using a model that 'batched' samples from the three OGTT collection times, diagnostic sensitivity was estimated as follows: 66.1% for HAPO research population and 48.4% for the 1305 women receiving routine antenatal care. If samples were not batched, but processed shortly after each blood sample was collected, then sensitivity increased to 81%.

Conclusion: Exploration of a range of clinical and laboratory scenarios using *in silico* modelling, showed that delaying the processing of pregnancy OGTT samples, using batched sample collection into fluoride tubes, causes unacceptable loss of GDM diagnostic sensitivity across two distinct population groups. This modelling approach will hopefully provide information that helps with final decision making around improved laboratory processing techniques.

1. Introduction

The 75 g Oral Glucose Tolerance Test (OGTT) is recommended in many countries for the diagnosis of gestational diabetes (GDM). While there are differing protocols with differing diagnostic cut-offs for GDM, the IADPSG (International Association of Diabetes and Pregnancy Study Group) recommends diagnosing GDM when any one of the following thresholds are exceeded: fasting glucose ≥5.1 mmol/L; one-hour glucose ≥10.0 mmol/L; or two-hour glucose ≥8.5 mmol/L [1].

Recently, several researchers have suggested that GDM is systematically underdiagnosed by routine clinical biochemistry laboratories. This is because the frequently used fluoride blood collection tubes allow pre-analytical glucose loss of glucose (subsequently described as 'glucose decay') to occur within the first few hours of collection. During routine collection, plasma separation is frequently delayed [2–4]. This delay in plasma separation is usually longer than the recommended maximum of half an hour [5]. This delay is exacerbated further by the common practice of batching the fasting, one-hour and two-hour OGTT samples so they can be analysed together, in part so results can be reported together with ease. This, in turn, leads to differing durations of glucose decay. Hence, systematic differences in measured glucose biases across the three OGTT samples are induced [2,3].

This *in silico* investigation aimed to determine the likelihood of misdiagnosis of GDM in the presence of pre-analytical induced bias in plasma

Abbreviations: GDM, gestational diabetes; OGTT, oral glucose tolerance test; HAPO Study, Hyperglycemia and Adverse Pregnancy Outcome; IADPSG, International Association of Diabetes and Pregnancy Study Group, fasting, one hour, two hour plasma glucose measurements from OGTT, respectively; CV, coefficient of variation; RG, research grade; TP, true positive; TN, true negative; FP, false positive; FN, false negative; ROC, received operator characteristic.

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glucose measurement, using two distinct clinical populations and different processing conditions, with a focus on fluoride collection tubes undergoing routine processing compared to research grade processing. Understanding the limitations associated with diagnostic testing is critical, either when making clinical decisions around interpretation of results, or when considering the likely impact of a change in laboratory processing.

2. Methods

2.1. Simulated cohort — unselected pregnant participants

A cohort of 1 million virtual test subjects was simulated using glucose distributions that were summarised in a prior study by Hypoglycaemia and Adverse Pregnancy Outcomes (HAPO) [6]. This is based on a large (n > 23.000) cohort of pregnant women who entered the HAPO study early in pregnancy and were tested using a 75 g OGTT test at 24 to 32 weeks gestation. It is important to note that in the HAPO study, the participants were not selected because of increased risk of GDM.

Fasting (G_0), one hour (G_1) and two hour (G_2) plasma glucose values were drawn from the distributions described by the following equations and pictured in Fig. 1:

$$G_0 = 3.9 + e^{\mathbb{N}(-0.8,0.6)} \tag{1}$$

$$G_1 = 2.1G_0 + \mathbb{N}(-1.9, 1.5) \tag{2}$$

$$G_2 = 0.2G_0 + 0.52G_1 - 2.3 + e^{\mathbb{N}(1.3,0.24)} \tag{3}$$

where: $\mathbb{N}(\mu, \sigma)$ is a normal distribution with a mean of μ and a standard deviation of σ.

These distributions were designed to create plausible OGTT results within ±2% of reported HAPO mean plasma glucose values ($G_0 = 4.5$ mmol/L, $G_1 = 7.4$ mmol/L, $G_2 = 6.2$ mmol/L), HAPO inter-sample correlation ($R_{0-1} = 0.38$, $R_{0-2} = 0.30$, $R_{1-2} = 0.68$) and standard deviations (0.4, 1.7, 1.3 mmol/L for G_{0-2} respectively).

Published intra-individual coefficient of variation (CV) on glucose measurement reported by the HAPO study was 4.4% [7]. Hence, to mimic results from the research-grade (RG) glucose analysis methods used by HAPO, the simulated glucose values were multiplied by a normal distribution of values, $G_{h,RG} = G_h\mathbb{N}(1, 0.044)$ for time (h) = 0, 1 and 2 h.

Both Daly et al. [2] and Carey et al. [3], demonstrated differences in pregnancy OGTT glucose results associated with delayed plasma separation and batching of the three OGTT samples, compared to research-grade methods equivalent to those utilised in HAPO. In the larger of the two studies by Daly et al., the mean plasma glucose differences between batched routine fluoride tubes and research-grade samples processing were −0.5, −0.4, and −0.2 mmol/L for G_0, G_1 and G_2 respectively [2]. In the Carey et al. data, the standard deviations in the differences between routine fluoride and research-grade were approximately 4% of the mean measured routine fluoride-tube value. Hence, routine processing of plasma glucose using fluoride collection tubes ($G_{0-2,F}$) were created with the following equations:

$$G_{0,F} = (G_{0,RG} - d_0)\mathbb{N}(1, 0.04) \tag{4}$$

$$G_{1,F} = (G_{1,RG} - d_1)\mathbb{N}(1, 0.04) \tag{5}$$

$$G_{2,F} = (G_{2,RG} - d_2)\mathbb{N}(1, 0.04) \tag{6}$$

where the decay values were assigned as $d_0 = 0.5$, $d_1 = 0.4$, $d_2 = 0.2$ mmol/L for routine batched fluoride-preserved samples.

A second HAPO-derived population was simulated using the assumption that processing was unbatched with a short venesection-to-analyser time causing glucose decay of 0.1 mmol/L for each of the three samples. Hence, for this case $d_0 = d_1 = d_2 = 0.1$ mmol/L.

2.2. Hybrid real/simulated cohort — high risk of GDM

Many health systems preferentially undertake pregnancy 75 g OGTTs in women considered to be at high risk of GDM, often as part of a two-step process of screening followed by diagnostic testing. We therefore assembled a second population, who were at higher risk of GDM compared to the HAPO derived virtual population discussed above. Local protocol is to screen for GDM using a 50 g glucose load and women who screen positive then undergo a diagnostic 75 g OGTT.

An alternative partially virtual cohort to the HAPO-based cohort in Section 2.1 was defined to mimic the outcomes of such a health screening strategy, by utilising a hybrid of real measurements from routine fluoride-tube methods with simulated research-grade measurements. The fluoride-tube glucose levels were transformed by the inverse functions of Eqs. (4)–(6) (Eqs. 7–9) to simulate the research grade distributions. The two data-sets were then compared to produce data on diagnostic sensitivity and specificity.

The research-grade simulated values were created with the Eqs. (7)–(9), which is turn are based on the glucose decay typically seen with batched samples. Both the real and simulated cohorts are pictured in Fig. 2.

$$G_{0,RG} = G_{0,F}\mathbb{N}\left(1, 0.04\sqrt{2}\right) + 0.5 \tag{7}$$

$$G_{1,RG} = G_{1,F}\mathbb{N}\left(1, 0.04\sqrt{2}\right) + 0.4 \tag{8}$$

$$G_{2,RG} = G_{2,F}\mathbb{N}\left(1, 0.04\sqrt{2}\right) + 0.2 \tag{9}$$

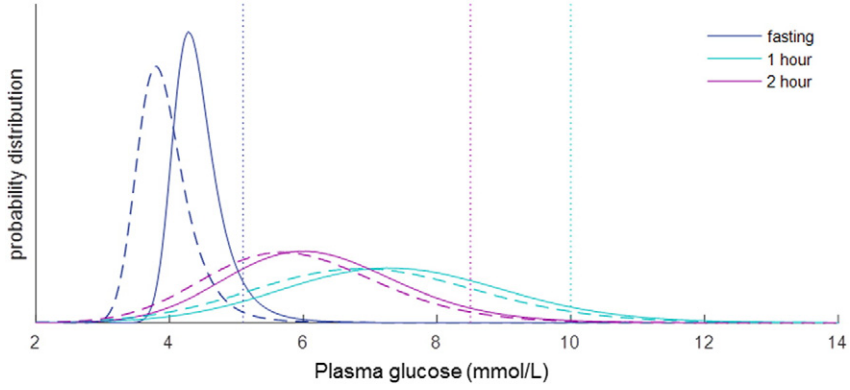

Fig. 1. The probability distributions for fasting, 1 and 2-hour plasma glucose in the simulated cohort. Solid lines indicate research-grade (Eqs. 1–3); dashed lines indicate routine fluoride tubes. (Eqs. 4–6; dotted lines show the diagnostic thresholds [1]).