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Case Report

The Risk from Anti-Thyroid Hormone Antibody Interference in Neonatal Congenital Hypothyroidism Screening

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Abstract: Neonatal congenital hypothyroidism screening is considered to be one of the most effective newborn screening strategies. Neonatal screening for congenital hypothyroidism involves the analysis of thyroid hormone and thyrotropin levels using an immunoassay based technique. Immunoassays are also prone to analytical problems such as assay interference. Immunoassays used for thyroid hormone measurement are known to be affected from anti-thyroid hormone antibody interference. This is the first reported case of interference presumably caused by anti-thyroid hormone antibodies transferred from mother to child during pregnancy, affecting the measurement of free-thyroxine in a cord-blood sample. We report a case of a full-term newborn with presumed anti-thyroid hormone antibody interference present in both cord blood and subsequent venous blood samples. In the context of a newborn screening programme based solely on thyroxine measurement, this is an important finding, since it has the potential to cause false negative screening results.

Keywords: neonatal hypothyroidism screening; anti-thyroid hormone antibody; interference; cord-blood

1. Introduction

Neonatal congenital hypothyroidism screening is considered to be one of the most effective newborn screening strategies adopted around the world. Early detection of cases and prompt initiation of treatment have been successful in preventing the complications associated with this neonatal condition. Screening procedures usually require the collection of a dried blood spot (DBS) sample obtained by heel prick sampling on days 3 to 5 following birth. The blood spot is analyzed for thyrotropin (TSH) and/or thyroxine (T4) concentrations [1]. The congenital hypothyroidism screening programme currently in place at our institution (Mater Dei Hospital, Malta), makes use of cord blood sample rather than a DBS. The screening strategy involves the simultaneous measurement of free thyroxine (FT4) and thyroid stimulating hormone (TSH) on each neonatal cord blood sample. Analysis of these hormones is performed using an immunoassay based technique using the Immulite 2000 (Siemens) analyzer

Immunoassays have a number of advantages such as automation and cost effective analysis. However, they are also prone to analytical problems such as assay interference. One type of interference which is usually associated with immunoassays arises from heterophilic antibodies. There are other forms of antibody-mediated interferences which may affect immunoassays, including free thyroxine (FT4) assays [2]. The FT4 assay is subject to interference by anti-thyroid hormone antibodies which particularly affects single wash competitive immunoassays [3]. John et al estimated the incidence of this form of interference to be 1 out of 2460 or 0.04% of all samples tested in his study [4]. Unpublished data obtained from a local study performed on the Immulite 2000 analyzer using the FT4 assay, shows an incidence of 0.15% in a general, non-pregnant population. Considering the number of thyroid

function tests performed daily, this translates into a significant number of samples exhibiting this form of interference. Whilst this may seem like a small amount, there is no actual data to describe the incidence of this situation. Moreover, it is within the remit of any screening programme to pick up 100% of cases, if possible.

2. Case Report

We report a case of a full-term female newborn with anti-thyroid hormone antibody interference present in both cord blood and subsequent venous blood samples. She was born with no obstetric complications to a healthy 34-year-old mother with no past medical history of note. The initial cord blood sample was submitted for routine neonatal congenital hypothyroidism screening testing. Results (Table 1) showed a TSH of 20.2 mIU/L (cut off for cord blood >12.8 mIU/L) and FT4 of 31.1 pmol/L (cut off for cord blood <10.8 pmol/L). Repeat analysis gave similar values, indicating the possibility of some form of assay interference affecting the analysis on the cord blood sample. A fresh venous sample was requested for further analysis and this was collected 5 days later. Results were as follows; TSH 2.52 mIU/L (reference range 0.3–3.0 mIU/L) and FT4 46.7 pmol/L (reference range 11–18 pmol/L).

The abnormal pattern of results, particularly the raised FT4, prompted the reflex testing using polyethylene glycol (PEG) treatment for the FT4 assay. This method is routinely used at our clinical chemistry lab to investigate discordant FT4 results in adults. The samples were divided in two aliquots with one of the aliquots subjected to PEG precipitation by adding equal volumes of 25% PEG. Treated samples were then vortexed, followed by centrifugation for 15 min at 3000 g [5]. The FT4 level was then measured from the supernatant of the PEG-treated sample. Positive interference was assessed on the basis of the residual difference obtained between the treated and untreated samples. A residual difference >2.38 was used as the cutoff at which positive interference is confirmed. This cutoff represents three standard deviation residual limits calculated from the regression equation obtained after plotting the FT4 values of untreated (x-value) against PEG-treated (y-value) euthyroid samples. Replacing x in the regression equation with the untreated FT4 value and subtracting this from the PEG-treated FT4 value gives the residual difference for the patient sample. The PEG-treated FT4 values obtained on both samples were 15.3 pmol/L and 27.7 pmol/L for cord blood and venous blood respectively. Both values produced a residual difference which is greater than the cutoff of 2.38. An aliquot from the venous sample collected on day 5 was also tested for FT4 using a different analytical platform, obtaining a result of 31.5 pmol/L which is comparable to that obtained from the PEG-treated sample. The second analytical platform chosen was Roche COBAS as it is a two-step assay and therefore less likely to be subject to interference from anti-T4 antibodies. The reason why two-step assays are less prone to this type of interference is because any interfering antibodies are washed off after the first wash step, thereby preventing interaction between the interfering antibodies and labeled-T4 analogue. A second venous sample was collected 11 days after the original cord blood specimen. Values obtained from this third sample were as follows; TSH 2.05 mIU/L and FT4 21.4 pmol/L (untreated sample), FT4 14.5 pmol/L (PEG-treated sample). It is also evident that the effect of this interference on the FT4 measurement decreased with each sample collected and this is represented from the gradual drop in the absolute percentage difference between treated and untreated samples (Table 2). This is to be expected, as the presumed maternal interfering antibodies are being cleared from the neonate's circulation. The progressive drop in the PEG-treated FT4 assay results in the neonate serves to exclude causes such as thyroid binding globulin, albumin or transthyretin for the abnormal results. These alternative sources of interference are also excluded since PEG treatment does not reduce the possible effect that these interferents can have on the FT4 measurement.

Table 1. Data showing residual difference between FT4 values pre and post 25% PEG treatment.

	* FT4 (x) pmol/L	* FT4 + 25% PEG (y) pmol/L	Residual Difference pmol/L ($(1.0028x + 0.4638) - y$)
Cord Blood	31.1	15.3	16.35
Venous Day 5	46.7	27.7	19.59
Venous Day 11	21.4	14.5	7.42

Method: * Immulite 2000, Note: Positive interference suspected if residual difference is >2.38.

Table 2. Data showing absolute % difference between FT4 values pre and post 25% PEG treatment.

	* FT4 pmol/L	* FT4 + 25% PEG pmol/L	Absolute % Difference (FT4 − FT4 + 25% PEG)/100
Cord Blood	31.1	15.3	16.35
Venous Day 5	46.7	27.7	19.59
Venous Day 11	21.4	14.5	7.42

Method: * Immulite 2000.

3. Discussion

This data shows that all three samples collected from the newborn were affected by anti-thyroid hormone interference which was demonstrable with 25% PEG treatment. This form of interference has been previously described in adult patients but is unexpected in newborns due to an immature immune system which is not developed enough to be able to mount an antibody response and thus produce these anti-thyroid hormone antibodies. This suggests that the presence of anti-thyroid hormone antibodies in the neonate is due to the transplacental transfer from mother to fetus. The baby was not being breast fed thus excluding the possibility of transfer of these same antibodies by means of breast milk. This is further supported by the drop in percentage difference seen in Table 2 reflecting the clearance of these interfering antibodies from the neonate's circulation. In our case, it is interesting to note that the mother's past laboratory results showed that a venous blood sample was submitted at week 6 of gestation during a routine antenatal review taken for TSH and FT4 levels. This also tested positive for PEG-precitable interferents on the FT4 assay, presumed to be anti-thyroid hormone antibodies. The FT4 result on the untreated maternal sample was 29 pmol/L (reference range 11–18 pmol/L) and after PEG treatment 13.3 pmol/L, whilst the TSH result was 1.65 mIU/L (reference range 0.3–3 mIU/L). It was not possible, either in the mother's serum or in the neonatal samples, to conclusively show that the actual interferent is anti-thyroid hormone antibody. However, it is known that PEG precipitation is able to identify cases of immunoglobulin mediated interference. Whilst this is regrettable, it should not distract from the basic message that interferents can exist in maternal sera that cross in utero to the neonate and subsequently can contribute to erroneous measurements in the FT4 assay. This has already been described in cases of macro-TSH complexes detected in both maternal and neonatal sera. This shows that it is possible for interfering antibodies to be passed from mother to child resulting in interference affecting hormone measurement from the newborn's blood [5–8].

4. Conclusions

To our knowledge, this is the first case report which describes positive FT4 interference caused by maternally transferred anti-thyroid hormone antibodies to a baby during pregnancy. This finding has several important implications on the outcome of newborn congenital hypothyroidism (CHT) screening programmes not least the potential risk of generating erroneous false negative screening test results.

While most screening programmes make use of a DBS specimen and not cord blood as their primary sample type, it cannot be excluded that such assay interference may also be present with DBS samples since in this report we were able to demonstrate that this particular interference was detectable

even in a peripheral venous sample collected a few days after birth. Various studies have shown that this form of interference is method dependent, with analogue based competitive methods and those assays with a single washing step being most affected [3,4]. Of note, the most common commercial diagnostic kit (DELFIA PerkinElmer) used in DBS thyroxine (T4) measurement uses a competitive method with a single washing step [9]. It is therefore possible that this interference may affect the DBS T4 result and further studies are required to investigate this issue in detail. Cord blood samples can sometimes be used as a primary sample for CHT screening in cases where there is maternal history of thyroid medication or family history of congenital hypothyroidism [1].

Although this form of interference is method dependent, it is important for any screening programme to bear in mind the possibility that such interference could affect either the DBS measurement or, as shown in this report, peripheral venous samples. In both instances this could result in false negative screening test results.

This case highlights that interferents exist for FT4 assays. Screening programmes that use only T4 measurements risk being victim to such interference without any possibility of detection. Failure to pick up interference carries the risk of missing a congenital case of hypothyroidism.

Whilst acknowledging that such cases are relatively rare, we feel that this case highlights why we feel that this is an added reason why screening programmes should adopt the simultaneous assessment of both TSH and T4. Naturally, the final decision lies with the service provider in conjunction with national health authorities.

Author Contributions: Ian Brincat and Gerald Buhagiar have both worked together in identifying the nature of the problem affecting this sample.

Conflicts of Interest: The authors declare no conflict of interest.

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