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Technical note

The effect of delay in collection and processing on RNA integrity in human placenta: Experiences from rural Africa

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ABSTRACT

This paper examines the relationship between time to processing and RNA quality in placentas collected from women in a field setting in rural Gambia. Placental samples were collected from the villages and transferred to the laboratory. RNA was extracted using Trizol and integrity assessed using the RNA integrity number (RIN). Values were inversely correlated with delay in processing. Expression levels of candidate genes increased with decreasing RIN. Normalising to a housekeeper gene removed this artefact. We propose a cut-off point of 90 min from delivery, after which samples cannot be used for gene expression analysis.

1. Introduction

The placenta is central to the healthy development of the fetus. Consequently, it has been studied extensively (see Ref. [1] for a recent review) However, isolating good quality material for molecular and biochemical analyses is not always straightforward, as many aspects of the collecting process can cause delays. Generally speaking, the consequences are not problematic for protein measurements or for transport studies, but can be more of a problem for measuring gene expression and mRNA levels [2].

Changes in the measured levels of mRNA can arise as a consequence of degradation. There is limited information on the impact of time between delivery of the placenta, processing in the laboratory and quality of the RNA. Here we examine the effect that delay can have on placental RNA quality and, secondly, the effect on mRNA expression measurement. We also determine whether using a housekeeper gene will remove any apparent effect of degradation on the measured levels of expression.

We measure RNA integrity using a Bioanalyser 2100 from Agilent Technologies, which calculates an "RNA integrity number" (RIN). The RIN ranges from 1 to 10, with 1 being the most degraded profile and 10 the most intact [3]. In this paper, we use the RIN to derive a cut-off value, below which RNA is of insufficient quality to be used in gene expression studies.

2. Material and methods

This study formed part of the ENID trial (ENID Trial; ISRCTN49285450). Full details are given elsewhere [4] and was approved by the joint Gambia Government/MRC Unit The Gambia Ethics Committee, (Project number SCC1126v2 & L2010.93). Written informed consent was obtained from all the women prior to recruitment into the study.

Women were recruited into the ENID Trial at <20 weeks gestation, and followed through pregnancy. Field workers resident in the villages attended the delivery. Immediately following delivery, the placenta was sealed in a sterile plastic bag and placed on ice, before transportation to the laboratory. The placentas were weighed and measured. Four washed sections (about 50 mg in weight) were fixed in RNALater® solution (Qiagen, UK). The processing times were noted and samples stored at −70 °C.

Total RNA was isolated from 50 to 100 mg of tissue by homogenising in cold Tri Reagent® (Helena Biosciences, UK) on wet ice using a Dounce homogeniser, precipitating in isopropanol and dissolving in DEPC treated water. RNA quantity and purity were measured spectrophotometrically by measuring OD 260/280 and OD 260/230 in a NanoDrop™ 2000c spectrophotometer (Thermo Scientific). RNA integrity was determined using Agilent 2100 Bioanalyzer® (Agilent Technologies) according to the manufacturer's instructions.

1 μg RNA was Dnase treated with Dnase I, Amp Grade® (Invitrogen) according to the manufacturer's instruction. Real time PCR reactions for transferrin receptor 1 (TfR1) (primer catalogue number QT00094850) were run using the 7500 Fast Real Time PCR system (Applied Biosystem) with ubiquitin (QT00234430) as the housekeeping gene. Gene expression was normalised to ubiquitin and expressed as $2^{-\Delta CT}$.

Abbreviation: RNA, ribonucleic acid; RT-PCR, reverse transcription polymerase chain reaction; ENID, early nutrition and immune development; TfR1, transferrin receptor 1.

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Single melt curves were obtained for all samples, indicating that a single PCR product was generated.

Statistical analysis was undertaken using linear regression. *P* values <0.05 were considered statistically significant.

3. Results

300 Women were recruited for this study, with normal pregnancies and deliveries occurring from October 2010 to November 2011. The mean (SD) age was 30.24 (6.2) years, parity 4.2 (2.5) and BMI 20.97 (3.3) kg/m^2.

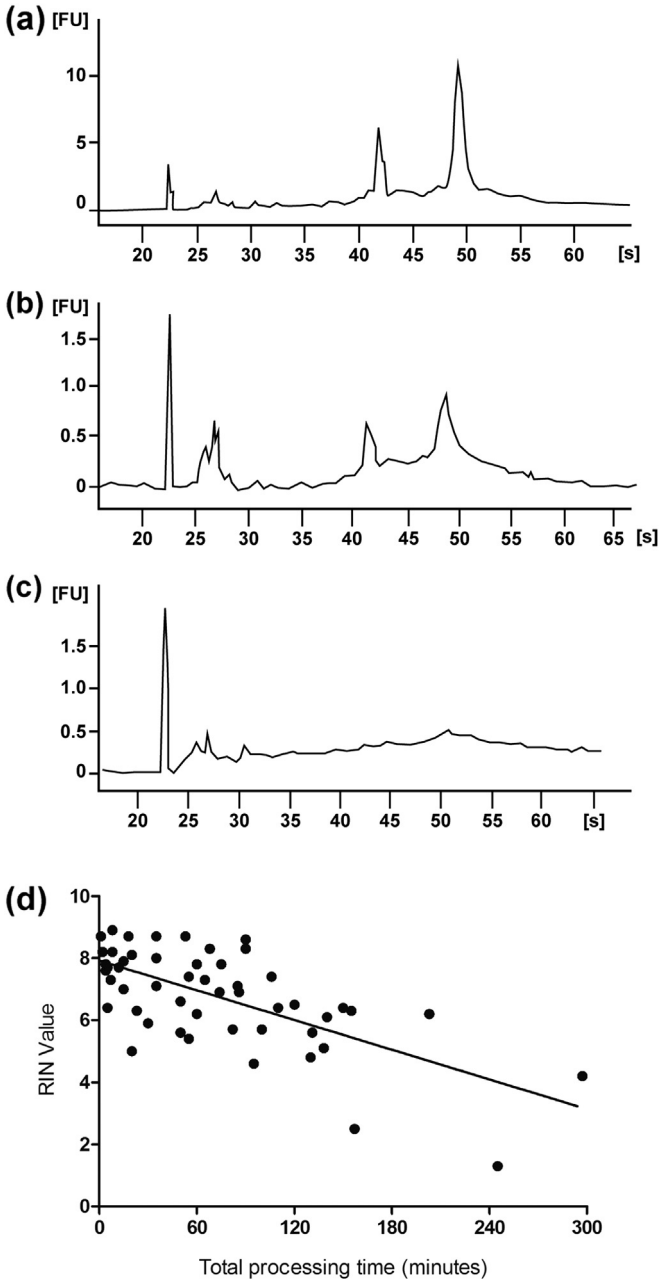

Fig. 1. The effect of delay in processing on the RNA integrity number (RIN). a, b and c show the electrophoretogram of RNA with RIN values of 8.3, 3.5 and 1.2 respectively. The degradation in the 18S and 28S peaks is apparent. (d) The relationship between delay in processing and RIN value is linear. Samples were collected as described in Materials and Methods and the RIN measured. The data are plotted against the time from delivery to processing. Analysis was by linear regression. R^2 was 0.44 and $P < 0.0001$.

Fig. 1a, b and c shows the spectra with RIN values of 8.3, 3.5 and 1.2, respectively. An inverse relationship was found between RIN and total placenta processing time (Fig. 1d). Time to processing did not alter yield of RNA.

To test whether the RIN value had an effect on measurement of mRNA levels, we performed RT-PCR for transferrin receptor (TfR1), and ubiquitin as a housekeeper. We used ubiquitin since expression is not affected by iron status (Gambling, L and McArdle, HJ unpublished results). As the RIN values decreased, there was an apparent increase in TfR1 mRNA levels (Fig. 2a). This effect was an artefact of degradation, and normalising the TfR1 mRNA with ubiquitin removed the association (Fig. 2b). The normalisation occurred irrespective of the RIN value, but since variation in expression levels was much greater as the RIN value decreased, samples with a RIN below 4.0 were excluded. This correlated to a delay in collection of about 90 min, and thus samples collected following a delay of more than 90 min were excluded from further analysis.

4. Discussion

This study demonstrates the importance of rapid collection and processing of placenta for analysis of gene expression. The results indicate that placenta RNA quality decreases with delay in processing time.

Given this, and given how difficult it is to collect placentas in challenging field situations, it is important to have an informed cut-off, below which samples cannot be used.

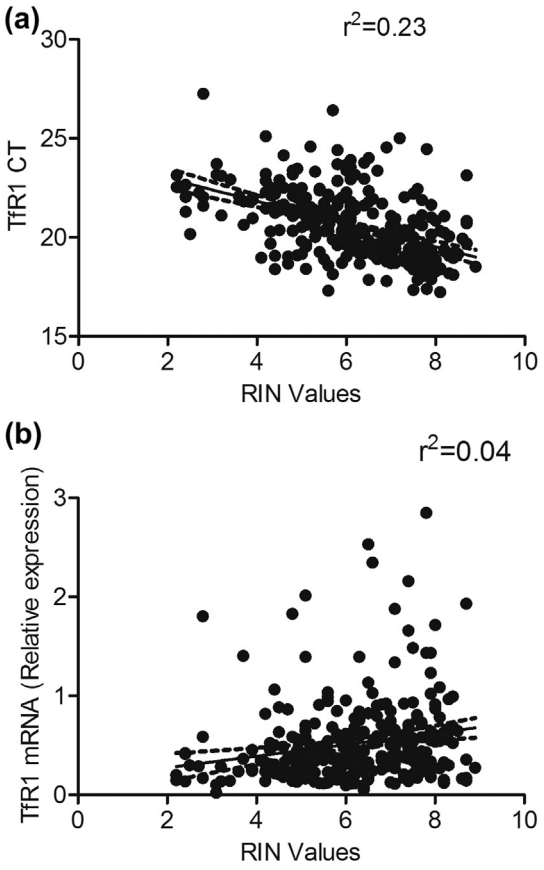

Fig. 2. (a) The effect of RNA integrity on apparent concentration of transferrin receptor 1 (TfR1) mRNA. TfR mRNA was measured as described in Materials and Methods and the C_t value measured. The data were analysed by linear regression. R^2 was 0.23 and $p < 0.0001$. (b) Normalising the TfR1 data to ubiquitin removes the effect of RIN on TfR1 expression. The data were analysed as described in Materials and Methods, and show no correlation to RIN ($R^2 = 0.04$).

There was an apparent increase in TfR1 levels in samples that had lower RIN values, which could lead to errors of interpretation [5]. Normalising the data to a housekeeping gene [6], removes the artefact. We can conclude, therefore, that measured levels of gene expression can be independent of the RIN, within certain limits. These limits are somewhat arbitrary, but below a RIN of 4, variability increases and risk of error becomes greater. Consequently, for future studies, and in agreement with other authors (12), we recommend using only samples processed within 90 min of delivery and a RIN value of 4.0 or greater, which have been held on ice or at 4 °C as soon as possible after delivery [5].

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SEM conceived and designed the original ENID Trial. SEM and HJM conceived the sub-study on placental development. MLJ coordinated all the field work, supervised all laboratory processing in The Gambia, conducted all experimental work in Aberdeen, performed statistical analyses and wrote the first draft of the paper. CK and LG supervised all laboratory work in Aberdeen. All authors contributed to the final version of the paper. None of the authors had a conflict of interest to report.

References

[1] Carter AM. Evolution of placental function in mammals: the molecular basis of gas and nutrient transfer, hormone secretion, and immune responses. Physiol Rev 2013;92:1543–76.
[2] Lanoix D, Lacasse Ae-A, St-Pierre J, Taylor S, Ethier-Chiasson M, Lafond J, et al. Quantitative PCR pitfalls: the case of the human placenta. Mol Biotechnol 2010;52:234–43.
[3] Schroeder A, Mueller O, Stocker S, Salowsky R, Leiber M, Gassmann M, et al. The RIN: an RNA integrity number for assigning integrity values to RNA measurements. BMC Mol Biol 2006;7:3.
[4] Moore S, Fulford A, Darboe M, Jobarteh ML, Jarjou L, Prentice A. A randomized trial to investigate the effects of pre-natal and infant nutritional supplementation on infant immune development in rural Gambia: the ENID trial: Early Nutrition and Immune Development. BMC Pregnancy Childbirth 2010;12:107.
[5] Fajardy I, Moitrot E, Vambergue A, Vandersippe-Millot M, Deruelle P, Rousseaux J. Time course analysis of RNA stability in human placenta. BMC Mol Biol 2009;10:21–30.
[6] Cleal JK, Day P, Hanson MA, Lewis RM. Measurement of housekeeping genes in human placenta. Placenta 2009;30:1002–3.