PEER REVIEW HISTORY

BMJ Paediatrics Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Can microRNA Profiles Predict Corticosteroid Responsiveness in Childhood Nephrotic Syndrome: A Study Protocol
AUTHORS	Patnaik, Saroj; Kumar, Pradeep; Yadav, Priya; Mittal, Anubha; PATEL, SAKSHI; Yadav, Mahendra; KANITKAR, MADHURI

VERSION 1 – REVIEW

REVIEWER	Reviewer name: Vishnu Swarup Institution and Country: All India Institute of Medical Sciences, New Delhi, India Competing interests: No competing Interest
REVIEW RETURNED	24-Jul-2018

GENERAL COMMENTS	The study protocol written by is well designed for further execution. However, following few minor points need to be addressed for better clarification of the manuscript: 1. Relative quantification of miR expression by qRT-PCR depends on the small nuclear RNA used as an internal control. There is no standard as to which internal control should be used for the normalization of qRT-PCR data, and inappropriate normalization can result in erroneous conclusions. Authors are requested to clarify about the internal controls to be used in their study. 2. Details of centrifugation speed/RCF etc. should be given for better clarity. Plasma requires one additional spin after separating cell pellet as there is always tiny cells left over after first spin which affect composition of plasma. 3. Accurate and quantitative estimation of miR profiles or specific miR expression levels and their correlation with a given condition is the key to fully understanding the function of miR biological processing. All of the current and new technologies have benefits and limitations to consider when designing miR studies. Results can vary across platforms, requiring careful and critical evaluation when interpreting findings. Authors are requested to clarify the troubleshooting strategies to tackle the conclusive findings.

REVIEWER	Reviewer name: Wei Zhao Institution and Country: Shandong University, China Competing interests: No
REVIEW RETURNED	12-Aug-2018

GENERAL COMMENTS	Dr Kumar presented a study protocol to evaluate the role of MicroRNA and metaboloproteomic expression in diagnostic of nephrotic syndrome in children. The study protocol is generally accepted for the study purpose, however the MicroRNA and metaboloproteomic study is still in a fundamental research stage.

	The clinical value still needed to be confirm. The BMJ pediatrics Open might not be suitable for such kind of study. A specialist journal or fundamental research journal is more suitable. In addition, the patients groups were divided to Steroid sensitive and steroid resistant nephrotic syndrome. However, in patients inclusion criteria, it required all case of nephrotic syndrome presenting for the first time without any immunomodulatory therapy. The authors should clarify this point.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Comment: Relative quantification of miR expression by qRT-PCR depends on the small nuclear RNA used as an internal control. There is no standard as to which internal control should be used for the normalization of qRT-PCR data, and inappropriate normalization can result in erroneous conclusions. Authors are requested to clarify about the internal controls to be used in their study.

Response: Thank you for the comment from the learned reviewer. We agree with the reviewer comments. We plan to use RNU6B as the internal control in our study for the qRT-PCR. Text marked in blue color on page number 12.

Comment: Details of centrifugation speed/RCF etc. should be given for better clarity. Plasma requires one additional spin after separating cell pellet as there are always tiny cells left over after first spin which affect composition of plasma.

Response: Thank you for the comment from the learned reviewer. We will remove any particular matter from plasma with additional final spin at 12000 rpm for 5-7 min at 4 degree. See para 1 methodology marked in red on page number 08.

Comment: Accurate and quantitative estimation of miR profiles or specific miR expression levels and their correlation with a given condition is the key to fully understanding the function of miR biological processing. All of the current and new technologies have benefits and limitations to consider when designing miR studies. Results can vary across platforms, requiring careful and critical evaluation when interpreting findings. Authors are requested to clarify the troubleshooting strategies to tackle the conclusive findings.

Response: Thank you for the comment from the learned reviewer. In this study we seek to subject the same sample at the discovery phase to next generation sequencing for miRNA, microchip microarray, and qRT-PCR. The latter two methods are dependent upon pre-existing miRNome panels while in NGS there is a possibility of novel miRNA detection. The method for ascertaining any novel miRNA by NGS will be as per existing guidelines on their characterization and the same has already been elucidated in the methodology section. Quantitative real-time reverse transcription PCR (qRT-PCR) will be the gold standard for determining the specific expression level. As on date it has been the gold standard method in published studies till date and we will be using this in the validation phase after design of customised primer sets. The troubleshooting strategies have been detailed under the data analysis and bioinformatics analysis headings under the material and methods section in full detail and are marked in yellow on page number from 13 to 16.

Reviewer: 2

Comment: The patients groups were divided to Steroid sensitive and steroid resistant nephrotic syndrome. However, in patient's inclusion criteria, it required all case of nephrotic syndrome presenting for the first time without any immunomodulatory therapy. The authors should clarify this point.

Response: Thank you for the comment from the learned reviewer. The inclusion criteria comprised of new cases (without any immunomodulation) as well as prevalent cases of nephrotic syndrome (in remission and off therapy for 4 weeks). The case recruitment is a continuous process and samples of consecutive patients with nephrotic syndrome encountered by us are being collected and genomic material is being archived. The determination and classification of patients into steroid resistance and steroid sensitivity for any initial case of nephrotic syndrome will be following exhibition to steroids as per standard guidelines on therapy and definitions of steroid resistance. Briefly, a child who received daily steroids for 4 weeks with 2 mg/kg of steroids and had persistent proteinuria will be classified as steroid resistant. The analysis of the miRNA profiles will be done at a later point after appropriate classification has been made. Cases will also be followed up for next 24 months to further phenotype the course of the disease as well as for detection of any late steroid resistance. Same has been marked in blue in the methodology section