Author's response to reviews

Title: Urinary amylase / urinary creatinine ratio (uAm/uCr) - a less-invasive parameter for management of hyperamylasemia.

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Author’s response to reviews: see over
Dear Dr. Peter O'Donovan

We are very grateful for providing us a chance to revise our manuscript to BMC Pediatrics. We tried to do our best to revise our manuscript. Therefore, we have changed (added) description in the text according to the reviewers’ comments and suggestions.

We have also responded point-by-point to the reviewers' comments and enclosed a list of the changes.

We are very happy if you re-consider our revised manuscript for publication in BMC Pediatrics at your earliest convenience.

Sincerely

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Response to reviewer’s comments:

Referee 1

1. In the conclusion the term patients with... should be added to the sentence: ...may be of use as an alternative to sAm during management of patients with hyperamylasemia.

   According to the reviewer’s suggestion, “patients with...” was added in Conclusion. The revised text is as follows; “uAm/uCr was found to significantly correlate with sAm, and therefore may be of use as an alternative to sAm during management of patients with hyperamylasemia.”

2. Figures 1 and 2 miss legends.

   Figure legends were added following References, prior to Tables in the original PDF file.

3. As discretionary, a revision of the Title, would be more appealing if "urinary amylase/urinary creatinine ratio" term be used. An example: "Urinary amylase/urinary creatinine ratio (uAm/uCr) - a less-invasive parameter for hyperamylasemia."

   According to the reviewer’s suggestion, the title was changed as follows; “Urinary amylase / urinary creatinine ratio (uAm/uCr) - a less-invasive parameter for management of hyperamylasemia.”
Response to reviewer’s comments:
Referee 2

1. As it is retrospective studies, why urinary amylase and creatinine were done in all those patients back from 1995. (because these investigations are not done routinely)

In the management of pancreatitis, change of the level of amylase is one of the most important information. We have used uAm (combined with sAm) as index of severity of pancreatitis since 1995. That was simply because we tried to decrease painful blood correction for sick children. On an empirical basis, we know that uAm/uCr is a good marker of hyperamylasemia. However, index using uAm had little scientific basis. In the present study, therefore, we investigated the concordance of uAm with sAm prospectively. To clarify these circumstances, we added the following text in Introduction; “Since 1990s, we have pursued possibility of using urine samples for management of pancreatitis, in order to decrease occasions of blood sampling. Index using uAm, however, had little scientific basis.”

2. Please provide a flow chart of data collection, how 804 samples of blood and urine collected from 128 patients. ACCR was calculated in 604 samples only.

To clarify the process of data collection, we added the text as follows in Methods. A flow chart of data collection (Fig 1) was also added.

“A flow chart of data collection is shown in Fig. 1. Firstly, 2931 urine samples of uAm obtained in our department were extracted from hospital databases. In these, 1255 samples were obtained with blood sampling measuring sAm on the same day of urine sampling. And in these, 806 samples were also taken with uCr simultaneously. One case of macroamylasemia (with 2 samples) was excluded. Diseases in the salivary glands were not included in the present study. Eventually, total of 804 urine and blood samples which contained uAm, sAm and uCr were collected from 128 patients.”
3. How UAm, Sam, and Ucreat was estimated, please mention the methods.

Explanations about measuring procedure of UAm, Sam and Ucreat were added in *Methods*, as follows: “Both sAm and uAm were determined by an enzymatic method using blocked p-nitrophenyl-α-maltoheptaoside as a substrate. uCr was determined by an enzymatic method involving creatinase, sarcosineoxidase and peroxidase.”

4. Adolescents age unclear as you have taken up to 29 years.

I am sorry for unclear classification of the age, especially in adolescents. In the present study, “adolescents” include cases ranging in age from 13 to 29 years old, and there were 22 twenties. To clarify age distribution, the text in *Result* was changed as below.

“Patients were divided into 4 groups: babies (<1 year old, n=18), infants (1-5 years old, n=266), schoolchildren (6-12 years old, n=330) and adolescents (>13 years old, n=190 including 22 twenties).”
5. How the urine samples were collected in infants and neonates, why it was morning sample, does it has any advantage over 24 hour urine sample? The recommended urine sampling technique is the second morning urine after voiding the night urine.

Detailed method of urine sampling was added, as follows: “The timings of sample collection were various and also independent from those of blood sampling. In neonates and infants, urine was sampled by using disposable Pediatric Urine Collector (Atom®).”

As the reviewer pointed out, the recommended urine sampling technique is the second morning urine after voiding the night urine. In the present retrospective study, however, the timing of urine sampling was not specified. This limitation was referred in Discussion as follows; “The method of urine sampling is also a limitation. The recommended urine sampling technique is the second morning urine after voiding the night urine, in order to avoid the influences of diurnal rhythms of enzyme excretion and collection errors [27]. In the present study, however, the time of day of urine sampling was not constant, and was also not constant in relation to the time of blood sampling. These issues of sample collection caused unavoidable measurement error, but they were considered acceptable by limiting the time of urine and blood sampling to the same day and by including a large number of samples.”

6. Can you specify non pancreatic disease as no. is 41.

We added details of “Non-pancreatic disease” in Result, as follows; “Non-pancreatic disease includes abdominal pain of unknown origin (n=14), ulcerative colitis (n=3), gallbladder stone (n=2), urinary tract infection (n=1) and SMA syndrome (n=1).”
7. References are older one only 3 references are after 2000, please provide newer one.

According to the reviewer’s suggestion, 4 references are added, as follows;

A description about trypsinogen-2 was also added in Discussion, as follows; “Urinary trypsinogen-2 strip test has also drawn attention recently for early detection of pancreatitis, but is not quantitative and less sensitive [23, 24].”