DOI: 10.1111/apt.15213

WILEY AP&T Alimentary Pharmacology & Therapeutics

Letter: studies of salivary pepsin in patients with gastro-oesophageal reflux disease

EDITORS,

We read with great interest the study by Race et al investigating the role of salivary pepsin in diagnosing gastro-oesophageal reflux disease (GERD).[1] Studies were performed on patients referred for consideration for anti-reflux surgery and asymptomatic healthy volunteers.

All subjects were intubated for the duration of the study and no control was used to show that intubation was not a cause of reflux episodes.[2,3] For good experimental design, we would recommend having a separate control group without intubation.

The evidence of validation for the pepsin ELISA has a series of flaws. The authors have failed to understand the significance of using antibodies and recombinant proteins, made specifically as research tools used primarily in Western blot which are difficult to interpret due to lack of MW markers to identify pepsin and are not recommended by the manufacturer for diagnostic procedures. The manufacturer demonstrated that the antibody, although monoclonal, had specificity for proteins other than human pepsin, detecting a protein the wrong MW for pepsin A at 45 kDa. The MW of pepsin is 34 kDa and pepsinogen is 41 kDa.[4]

The data on the ELISA were not robust; the amount of pepsin present in gastric juice is approximately 0.9 mg/mL.[5] Reflux into the airways would be diluted with a wide concentration range. The ELISA test results gave a dynamic range of 0-100 ng/mL, with 90% less than 50 ng/mL and an average of 20 ng/mL. The expected range was not present with the ELISA, suggesting either pepsin was not being detected or a high-dose hook effect had been exceeded. However, Peptest gave a range of 0 to >500 ng/mL with an appropriate standard deviation. This is more typical of a single analyte being measured.

Literature presented describing the use of salivary pepsin as a diagnostic marker for GERD was used to argue that there were no differences between patients and controls—this was misleading. Kim et al[6] stated that all the healthy volunteers were negative for pepsin and Lannella et al[7] stated that subjects belonging to the control group were pepsin negative, and likewise, Birtic et al[8] came to similar conclusion. In another paper referenced by Race et al, they stated no difference between controls and GERD patients suggesting low reproducibility; this was not shown or stated in the paper by Du et al.[9]

Finally, in response to the comment on the expression of pepsin in the tongue used as an argument for the presence of pepsin in saliva was misleading. The evidence presented for pepsinogen expression referenced the Fantom 5[9] project. The Human Expression Atlas reports 9 studies on tissue expression of pepsinogen 3. Only the Fantom 5 project predicts any tongue expression. The other 8 had none. The level reported by Fantom 5[10] is four transcripts per kilo base millions and the ovaries is 14 141, a tissue not recognised as a major secretor of pepsin. Consequently, the level of expression reported in the tongue could not account for levels measured in saliva.

ACKNOWLEDGEMENTS

Declaration of personal interests: ADW is employed by RD Biomed Limited and PWD is a director of RD Biomed Limited.

FUNDING INFORMATION

None.

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LINKED CONTENT

This article is linked to Race et al and Corfe et al papers. To view these articles, visit https://doi.org/10.1111/apt.15138 and https://doi.org/10.1111/apt.15225.

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DOI: 10.1111/apt.15225

Letter: studies of salivary pepsin in patients with gastro-oesophageal reflux disease. Authors' reply

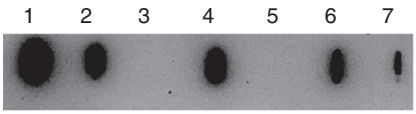
EDITORS,

We are grateful for the opportunity to respond to Dettmar et al[1] who raise a number of concerns relating to our report[2]:

First, Dettmar et al question our use of intubation, suggesting that this may in and of itself cause reflux episodes. Whilst some have suggested nasogastric intubation may promote reflux, studies are conflicting.[3] However, our results clearly show pathological levels of reflux in patients and physiological levels in controls. Furthermore, our methodology allowed us to look at the temporal relations between the appearance of salivary pepsin and reflux episodes but none were apparent. Nonetheless, during our method development the researchers (who are asymptomatic) did test their own saliva without intubation, some samples of which exhibited detectable cross reaction with the anti-pepsin antibody (Panel A below).

Second, it is suggested the validation of our ELISA is flawed. We believe our assay methodology is both transparent and robust. We sourced a monoclonal antibody (which cites ELISA amongst its recommended applications[4]) and, using an independently sourced antigen, we showed using western immunoblot that there is a single primary cross-reaction with a high signal-to-noise ratio (Supplement Section 1) and that when used in ELISA it gave a linear response range as far as 100 ng, with a limit of detection of 10 ng (*ibid*). We showed that the quantitation of pepsin in saliva samples exhibits a

(A)



(B)

Group	n	>/=1 positive	3 positive
GERD	122	111	16
Control	35	15	4

FIGURE 1 Panel A saliva samples from researchers were taken, and quantified for total protein. 5 μg of total protein was applied to each well of a dot-blot system and were immunoprobed as described for western blotting. Five of seven samples exhibited cross reactivity with the anti-pepsin monoclonal antibody. Panel B extracted data from Du et al comparing the number of positives using Peptest in one and three independent repeats in GERD and Control. There is no significant difference between the proportion of controls and GERD patients with three positive tests. There is a highly significant difference between the number of GERD patients with at least one positive test and three positive tests ($P < 0.001$, χ^2)