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Original article

The characteristics of salivary pepsin in patients with severe motor and intellectual disabilities

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Abstract

Purpose: The aim of the present study was to determine the utility of measuring the salivary pepsin level (SPL) as an objective assessment of gastroesophageal reflux disease (GERD) in severe motor and intellectual disabilities (SMID) patients.

Subjects and methods: This prospective study included 26 SMID patients who underwent simultaneous 24-h multichannel intraluminal impedance pH measurement (pH/MII) and SPL evaluation. The enrolled patients were divided into GERD (+) or GERD (−) groups according to the pH/MII findings. The age, gender and pH/MII parameters were compared between the two groups. A correlation analysis was also conducted for the SPL following early-morning fasting and post-enteral feeding and the age, gender, presence of gastrostomy and tracheostomy and pH/MII parameters. The SPL was compared between the two sampling groups.

Results: Fifteen patients were classified as GERD (+), and 11 patients were classified as GERD (−). The mean SPL following early-morning fasting and post-enteral feeding among all patients were 104.3 (median: 38, 25th and 75th percentile: 12, 361) ng/ml and 222.2 (median: 152:0, 500) ng/ml, respectively. Regarding positivity, 76.9% and 73.1% of SPL values in early-morning fasting and post-enteral feeding SMID patients, respectively, were positive (≧16 ng/ml). The SPL following early-morning fasting demonstrated a weak but significant positive correlation with age. In contrast, we noted no correlation between the pH/MII parameters and the SPL for either the early-morning fasting or post-enteral feeding patients, and no significant difference in the SPL was observed between the GERD (+) and (−) patients.

Conclusions: The present study showed that a high proportion of SMID patients had a relatively high SPL, regardless of the presence of GERD. The SPL in SMID patients might be affected by several distinctive factors in addition to gastroesophageal reflux.

Keywords: Salivary pepsin; Severe motor and intellectual disabilities; Gastroesophageal reflux disease

1. Introduction

Severe motor and intellectual disabilities (SMID) patients account for the majority of patients suffering from gastroesophageal reflux disease (GERD) who require anti-reflux surgery [1]. While 24-h pH monitor-

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ing is widely used as the gold-standard method for evaluating GERD, this conventional examination is unable to detect non-acidic refluxes, and false-negative results have been reported at rates of 15–30% [2]. Recently, 24-h multichannel intraluminal impedance pH measurement (pH/MII) has been established as a pH-independent measurement tool [3–6]. The main advantage of this novel technique is that the pH of both acidic and non-acidic reflux episodes can be simultaneously detected using a multiple-impedance catheter with integrated pH sensors. There is also a good correlation between the pH/MII and manometry or videofluoroscopy in healthy subjects as well as in patients with GERD [3]. There have been previous reports evaluating pH/MII in SMID patients. In 2006, Del Buono et al. reported that in 16 SMID children with 12-h impedance recordings, more than half of the reflux events were non-acidic and would have gone undetected by conventional pH measurement [4]. We previously reported on the utility of pH/MII for detecting GERD subtypes that could not be detected by 24-h pH monitoring and suggested that pH/MII be considered the go-to tool for GERD testing, especially in SMID patients [5,6]. Moreover, Tanaka et al. were reported that the postoperative reflux parameters such as pH reflux index, acid clearance time, bolus exposure index, and the number of acidic reflux episodes significantly decreased ($P < 0.05$) compared with the preoperative ones in SMID patients using 24 h pH/MII [7]. Evaluating pH/MII were also effective tool for detecting GERD in SMID patients. However, despite the usefulness of pH/MII for diagnosing GERD, this technique is often invasive and expensive due to the requirement of nasogastric intubation for a long time, which is uncomfortable and inconvenient, especially for SMID patients.

Salivary pepsin, which is produced in the stomach, was recently introduced as a new biomarker for the diagnosis of GERD in healthy subjects [8–10]. In a single adult study evaluating proximal oesophageal reflux by conducting simultaneous salivary pepsin and pH measurements, the sensitivity and specificity of salivary pepsin for predicting proximal oesophageal reflux was reported to be 75% and 91%, respectively [10]. Furthermore, in a similar study, the sensitivity and the specificity of a positive salivary pepsin value for the diagnosis of GERD-related symptoms was reported to be 77.6% and 63.2%, respectively [8]. However, no reports have yet described the relationship between the presence of salivary pepsin and GERD in SMID patients.

The aim of the present study was to determine the utility of measuring the salivary pepsin level (SPL) as an objective assessment of GERD in SMID patients.

2. Patients and methods

2.1. Patients

This prospective study included 26 SMID patients (male/female: 17/9; mean age: 15.0 years, median: 14.0 years, 25th and 75th percentile: 4, 19.5 years). Regarding the causal disorders of SMID, 3 patients had a genetic anomaly, 1 had a chromosomal anomaly, 2 had adrenoleukodystrophy, 8 had suffered cerebral damage in the neonatal period, 11 had suffered cerebral damage in infancy or later and 1 was unknown. Nineteen patients were fed via a nasogastric tube, and seven were fed via a gastrostomy tube. Fourteen patients underwent tracheostomy. Pharmacotherapies for GERD, such as the administrations of prokinetics, laxatives and Japanese herbal medicine, were discontinued at least three days before the patients entered the study. The study protocol was approved by the Kurume University Ethical Committee (No. 13179). Informed consent was obtained from the families before the participants were enrolled in the study.

2.2. 24-h multichannel intraluminal impedance pH measurements

A multiple intraluminal impedance catheter (outer diameter, 2 mm) with 2 pH antimony electrodes and 7 impedance electrodes (Sandhill Scientific, Inc., Highlands Ranch, CO, USA) was used. The catheter was inserted transnasally through the oesophagus, and the pH sensor placement was confirmed by radiography. The impedance data were automatically evaluated using the BioVIEW analysis software program, and each tracing was manually reviewed by the same author (SF).

Liquid reflux was defined by pH/MII as a fall in the impedance $\geq$50% from baseline in at least two consecutive channels in an aboral direction. Each type of reflux was defined as follows: acidic reflux was diagnosed in cases associated with a pH drop to $\leq$4, non-acid reflux was diagnosed in cases associated with a nadir pH value >4. The pH index (pHI) was defined as the percentage of time that the pH was $\leq$4.0. We defined 4.0% as the upper cut-off value in accordance with the definition outlined in the NICE guidance 2015 [11]. The bolus exposure index (BEI) was defined as the percentage of time with retrograde movement of intraluminal oesophageal material. We defined 1.4% as the upper cut-off value [12]. Pathological GERD was defined as a case in which the pHI exceeded 4.0% or the BEI exceeded 1.4%. The numbers of total reflux episodes (TRE) (total/acid/non-acid) and proximal reflux episodes (PRE) (total/acid/non-acid) were also evaluated. GERD in all patients was evaluated and diagnosed in accordance with the above pH/MII

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parameters, namely pHI, BEI (total/acid/non-acid), TRE (total/acid/non-acid) and PRE (total/acid/non-acid).

2.3. Salivary pepsin analysis

Early-morning fasting and post-enteral feeding saliva samples were collected from each patient's mouth into tubes containing 0.5 mL of 0.01 M citric acid. Each collected sample was refrigerated at 4 °C and analysed within 2 days of collection. The collection tubes containing the saliva samples were centrifuged at 4000 rpm for 5 min. If a clear supernatant was not present, the samples were centrifuged again, and 80 μL from the surface layer of the centrifuged sample was drawn up into an automated pipette. The 80-μL sample was transferred to a screw-top microtube containing 240 μL of migration buffer solution. This sample was mixed with a vortex mixer for 10 s. A second pipette was used to transfer 80 μL of the sample to the circular well of a lateral flow device (LFD) containing two unique human monoclonal antibodies: one to detect and one to capture pepsin in the saliva sample (Peptest™; RD Biomed Ltd., Kingston upon Hull, UK). If pepsin was present in the saliva, a second line appeared under the letter T (arrow) between 5 and 15 min after applying the saliva sample (Fig. 1). The lower limit for the pepsin level with this test kit was 16 ng/mL (as determined by the manufacturer), and a pepsin level below this threshold was represented as 0 ng/mL. The lower limit for pepsin level quantitation was 25 ng/mL, and any data between 16 and 25 ng/mL were represented as 16 ng/mL. The upper limit for pepsin level quantitation was set at 500 ng/mL. The ratio of the SPL following post-enteral feeding/early-morning fasting (SPL ratio) were revealed. The upper limit for SPL ratio was set at 500.

All patients underwent the pH/MII analysis and were designated as pathological GERD (GERD (+)) or other (GERD (−)) patients based on the pH/MII results. Correlation analyses were conducted between the pH/MII parameters and age or gender. Thereafter, the gender, age, presence of gastrostomy and tracheostomy and pH/MII parameters were compared between the two GERD groups. The SPL following were also compared between the patients sampled early-morning fasting and those sampled after post-enteral feeding. A second correlation analysis was then conducted between the SPL following early-morning fasting and post-enteral feeding and the age and pH/MII parameters. The SPL following early-morning fasting and post-enteral feeding were compared between the two GERD groups. Finally, the gender, age, presence of gastrostomy and tracheostomy and pH/MII parameters were compared between between SPL >1 and SPL ≦1.

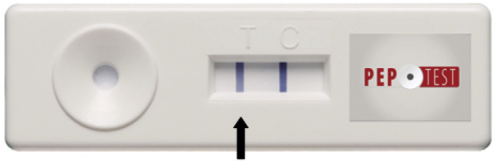

Fig. 1. If pepsin was present in the saliva, a second line appeared under the letter T (arrow) between 5 and 15 min after applying the saliva sample.

2.4. Statistical analyses

All statistical analyses were performed using the JMP software package (SAS, Cary, NC, USA). Data were presented as the mean ± standard deviation, median and range. Comparison analyses of the SPL between the patients sampled after early-morning fasting and those sampled after post-enteral feeding or between the GERD (+) and GERD (−) patients were conducted using a Pearson's chi-squared test and a Mann–Whitney *U* test. Correlations analyses between the pepsin concentration and pH/MII parameter variables were assessed with Spearman's rank sum tests when appropriate. A *p*-value of <0.05 was considered to indicate a statistically significant difference.

3. Results

In the GERD-related parameters analyses using pH/MII, the mean pHI, BEI (total), TRE (total) and PRE (total) of all patients were 7.76% (median 2.6%; 25th and 75th percentile: 0.35%, 9.75%), 1.11% (0.9%; 0.38%, 1.7%), 46.9 (48.5; 21.8, 66.0) and 20.0 (15;3.5, 30.7), respectively. Furthermore, the BEI (acid/non-acid), TRE (acid/non-acid) and PRE (acid/non-acid) were 0.91% (0.75%; 0.2%, 1.4%)/0.18% (0.1%; 0%, 0.23%), 36.1 (37.5; 13.5, 54.8)/10.7 (9; 3.5, 17) and 13.6 (8.0; 2, 16)/4.31 (1.0; 0, 5.5), respectively.

Regarding a relationship between the pH/MII parameters and age or gender, no correlation was observed between the pH/MII parameters and age or gender. Fifteen patients were classified as GERD (+), and 11 patients were classified as GERD (−) according to the pH/MII findings. On comparing the age, gender and pH/MII parameters between the GERD (+) and (−) patients, there were no significant differences in the age or gender between the two groups. In contrast, however, the pHI, BEI (total/acid), TRE (total/acid) and PRE (total/acid) were significantly higher in GERD (+) patients than in GERD (−) patients ($p < 0.001$, $p < 0.001/p < 0.001$, $p < 0.001/p < 0.001$ and $p = 0.015/p = 0.016$ respectively) (Table 1).

In the investigation of the salivary pepsin, the mean SPLs following early-morning fasting and post-enteral feeding among all patients were 104.3 ng/ml (38.0 ng/ml;12 ng/ml, 36.1 ng/ml) and 222.2 ng/ml (152 ng/ml; 0 ng/ml, 500 ng/ml), respectively. Regarding positivity,

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Table 1
The comparisons of age, gender, gastrostomy, tracheostomy and pH/MII parameters between GERD (+) and (−) groups.

	GERD(−) ($n = 11$)	GERD(+) ($n = 15$)	p value
Gender (M/F) (no.)	7/4	10/5	0.873
Age (years)	14(4, 18)	14(4, 22)	0.735
Gastrostomy (%)	45.5	13.3	0.068
Tracheostomy (%)	54.6	53.3	0.951
pHI (%)	0.8(0, 2.3)	9.5(1.9, 20.1)	<0.001
BEI (total) (%)	0.4(0.2, 0.7)	1.6(1.1, 2.0)	<0.001
BEI (acid) (%)	0.2(0, 0.6)	0.7(1.2, 1.9)	<0.001
BEI (non-acid) (%)	0.1(0, 0.2)	0.2(0, 0.3)	0.274
TRE (total) (no.)	23(11, 41)	64(48, 71)	<0.001
TRE (acid) (no.)	12(2, 27)	49(35, 65)	<0.001
TRE (non-acid) (no.)	9(2, 14)	11(4, 22)	0.298
PRE (total) (no.)	4(2, 16)	22(12, 46)	0.015
PRE (acid) (no.)	2(0, 7)	6(11, 35)	0.016
PRE (non-acid) (no.)	1(0, 4)	1(0, 9)	0.502

GERD: gastroesophageal reflux disease, pHI: pH Index, M/F: male/female, BEI: bolus exposure index, TRE: the number of total reflux episodes, PRE: the number of proximal reflux episodes.
Date displayed as median and 25th, 75th percentile.

76.9% and 73.1% of SPL values in early-morning fasting and post-enteral feeding SMID patients were positive, respectively, and 88.4% of SPLs were positive in either or both early-morning fasting or post-enteral feeding.

The SPL following early-morning fasting demonstrated a weak but significant positive correlation with age ($r = 0.450$, $p < 0.05$), while there were no significant differences between the SPL following early-morning fasting by gender or presence of gastrostomy and tracheostomy. Furthermore, there was no significant correlation between SPL following post-enteral feeding and any clinical parameters, nor was there any significant correlation between the pH/MII parameters and the SPL of either the early-morning fasting or post-enteral feeding patients (Table 2).

The SPL following post-enteral feeding was significantly higher than that following early-morning fasting ($p < 0.05$) (Fig. 2). The mean SPL in early-morning fasting GERD (+) and GERD (−) patients was 150.5 ng/ml (51 ng/ml;16 ng/ml, 254 ng/ml) and 41.4 ng/ml (32 ng/ml;0 ng/ml, 74 ng/ml), respectively, while the mean SPL in post-enteral feeding GERD (+) and GERD (−) patients was 259.9 ng/ml (183 ng/ml;0 ng/ml, 500 ng/ml) and 131.5 ng/ml (82 ng/ml;0 ng/ml, 328 ng/ml), respectively. Regarding positivity, 80% and 72.7% of SPL values following early-morning fasting were positive among GERD (+) and GERD (−) patients, respectively. No significant differences were observed in the SPL values following early-morning fasting or post-enteral feeding between the GERD groups (Fig. 3a and b).

Fourteen patients were classified as SPL ratio >1, and 12 patients were classified as SPL ratio ≦1. On comparing the age, gender and pH/MII parameters between SPL ratio >1 and SPL ratio ≦1, TRE (total) and TRE (acid) were significantly lower in SPL ratio >1 patients than in SPL ratio ≦1 patients ($p = 0.009$ and $p = 0.019$ respectively). However, there were no significant differ-

Table 2
The correlation analyses between age and pH/MII parameters and SPL in SMID patients.

	Early-morning fasting		Post enteral feeding	
	Variables	p value	Variables	p value
Age (years)	0.459	0.021	0.051	0.805
pHI (%)	−0.163	0.427	0.086	0.679
BEI (total) (%)	0.084	0.685	−0.015	0.942
BEI (acid) (%)	0.128	0.535	0.063	0.760
BEI (non-acid) (%)	−0.010	0.958	−0.357	0.073
TRE (total) (no.)	0.145	0.481	−0.288	0.153
TRE (acid) (no.)	0.150	0.464	−0.207	0.311
TRE (non-acid) (no.)	−0.012	0.952	−0.161	0.432
PRE (total) (no.)	0.125	0.575	−0.124	0.546
PRE (acid) (no.)	0.068	0.739	−0.123	0.548
PRE (non-acid) (no.)	−0.186	0.363	−0.160	0.436

pH/MII: 24 h multichannel intraluminal impedance pH measurements, SPL: salivary pepsin level, SMID: severe motor and intellectual disabilities, pHI: pH Index, BEI: bolus exposure index, TRE: the number of total reflux episodes, PRE: the number of proximal reflux episodes.

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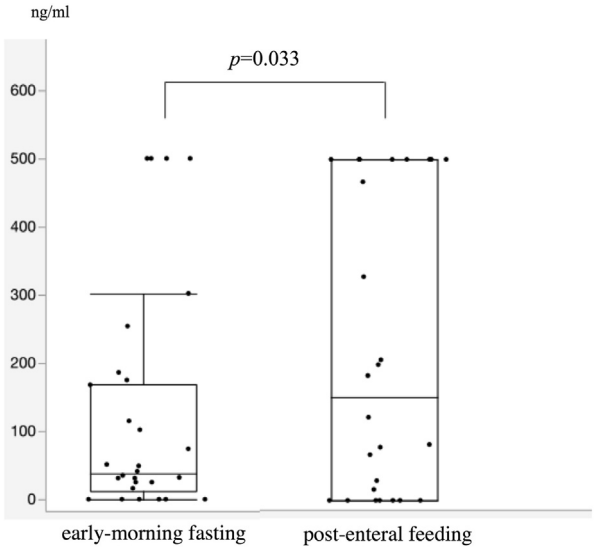

Fig. 2. The SPL of post-enteral feeding was significantly higher than that of early-morning fasting ($p = 0.033$).

ences in the age, gender and the other pH/MII parameters between the two groups (Table 3).

4. Discussion

Recently, the detection of pepsin in the saliva or sputum has been proposed as a non-invasive method for diagnosing GERD [8,9] and its related diseases, such as otitis media with effusion [13] and aspiration pneumonia [14]. A salivary pepsin analysis for the diagnosis of GERD has been introduced as a convenient, office-based, non-invasive, quick and inexpensive technique, unlike other available tools [9]. To our knowledge, the present study is the first clinical investigation exploring simultaneous measurement of SPL and 24-h pH/MII in SMID patients.

Interestingly, in the present study, the prevalence of the SPL in early-morning fasting SMID patients was higher (76.9%), regardless of the presence of GER, than that previously reported by Saritas Yuksel et al., who reported SPL in 6/51 (12%) control samples and 13/58 (22%) GERD patients [9]. Furthermore, Hayat et al. reported that 33/87 (37.9%) healthy control samples and 45/58 (77.6%) samples from GERD patients were positive for SPL [8]. In the present study, we detected positive SPL values in 72.7% of GERD (−) patients, and the presence of SPL in early-morning fasting SIMD patients did not correlate with any pH/MII parameters. Taken together, these findings suggest that that GER content containing highly concentrated gastric pepsin is pooled in the mouth for long periods in SMID patients due to several distinctive characteristics, such as their swallowing dysfunction, impaired oesophageal clearance, long duration spent in a recumbent position and reduced salivary secretion. These conditions specific to SMID patients might induce discrepancies in findings between the present study and the GERD analyses in previous reports in neurologically normal adults.

For the above reasons, unlike in healthy subjects, the SPL in the SMID patients might be affected by a number of factors in addition to GER. Furthermore, this condition might increase the risk of aspiration pneumonia, chronic otitis and tooth erosion, although further studies will be required. On comparing the SPL following early-morning fasting and post-enteral feeding, we found that the SPL following post-enteral feeding patients was significantly higher than the early-

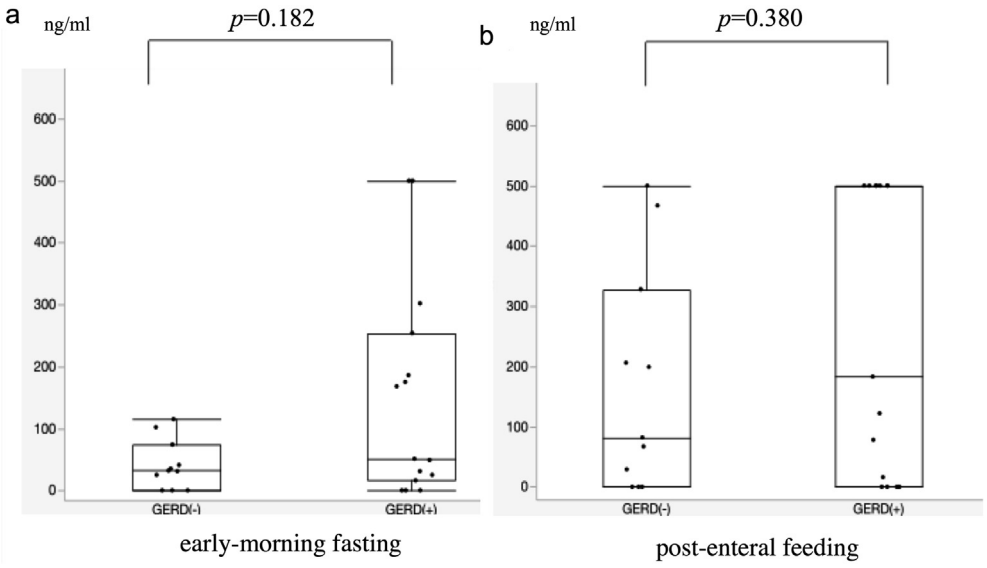

Fig. 3. No significant differences were observed in the SPLs following early-morning fasting (a) and post-enteral feeding (b) between the GERD (+) and GERD (−) patients.

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Table 3
The comparisons of age, gender, gastrostomy, tracheostomy and pH/MII parameters between SPL ratio >1 and ≦1 groups.

	SPL ratio >1($n = 14$)	SPL ratio ≦1($n = 12$)	p value
Gender (M/F) (no.)	10/4	7/5	0.484
Age (years)	11(3.75, 18.3)	14.5(7.5, 23.3)	0.290
Gastrostomy (%)	35.71	16.67	0.268
Tracheostomy (%)	50	58.3	0.671
GERD (%)	50	66.7	0.389
pHI (%)	2.1(0.18, 12.7)	3.4(0.7, 9.6)	0.718
BEI (total) (%)	0.7(0.28, 1.75)	1.25(0.63, 1.65)	0.455
BEI (acid) (%)	0.7(0.18, 1.3)	0.8(0.6, 1.4)	0.535
BEI (non-acid) (%)	0.1(0, 0.2)	0.2(0.1, 0.3)	0.131
TRE (total) (no.)	33(12.8, 51.3)	65(50.3, 74.8)	0.009
TRE (acid) (no.)	19.5(7, 37)	51.5(40.5, 63.5)	0.019
TRE (non-acid) (no.)	4.5(2, 15.3)	11.5(5.75, 17)	0.380
PRE (total) (no.)	12(2, 17.8)	21.5(7.75, 48.5)	0.063
PRE (acid) (no.)	5.5(1.5, 12.8)	10(4.5, 31)	0.206
PRE (non-acid) (no.)	0.5(0, 4.75)	1.5(0.25, 8)	0.287

GERD: gastroesophageal reflux disease, pHI: pH Index, M/F: male/female, BEI: bolus exposure index, TRE: the number of total reflux episodes, PRE: the number of proximal reflux episodes.
Date displayed as median and 25th, 75th percentile.

morning fasting patients. Hayat et al. reported that the prevalence of positive pepsin samples and concentration of pepsin in saliva were significantly lower in the morning waking samples compared to postprandial samples, in controls and patient groups [9]. This might be explained by increased postprandial gastric pepsin concentration and volume of reflux [15]. Based on the results, only postprandial saliva sampling would be recommended in the clinical office setting. However, although the SPL ratio value should increase over one, 12 patients were classified as SPL ratio ≦1. Moreover, TRE (total) and TRE (acid) were significantly lower in SPL ratio >1 patients than in SPL ratio ≦1 patients and the other pH/MII parameters were not significantly different between 2 groups. TRE which did not involve with pathological GERD parameters such as short term reflex episodes and several distinctive characteristics in SMID might be the bias of SPL positivity in GERD (−) patients.

Regarding the relationship between the SPL and age, Feldman et al. reported that gastric acid and pepsin output rates were similar in young (age range, 18–34 years) and middle-aged (age range, 35–64 years) individuals [16], and Hayat et al. reported that the salivary pepsin concentration was not significantly different between younger (<55 years) and older patients (≥55 years). In the present study, the fasting SPL showed a significantly positive correlation with age, which was not consistent with previous findings. Another study of subjects ≥18 years of age suggested that unstimulated oral salivary flow rates were significantly lower in older adults than in younger ones, regardless of medication usage [17]. It was supposed that the oral salivary flow decreased with age in SMID patients due to non-oral intake, which might consequently elevate the concentration of SPL. The present results suggest that older SMID patients might be at risk of aspiration pneumonia and tooth erosion as they age, although there have been no reports of a relationship between age (including young age) and SPL in healthy subjects. Further studies are needed to characterize the relationship between age and SPL in SMID patients.

In summary, the present study showed that more than two-thirds of the SPL values in SMID patients were markedly high, contrasting with previous findings in healthy subjects, and the values were positively correlated with age. However, no significant differences were observed in the SPL between GERD (+) and GERD (−) patients despite the presence of significant differences in the pH/MII parameters between the two groups.

Several limitations associated with the present study warrant mention, including the small number of subjects and the wide age distribution compared with previous reports [7,8] and the lack of established normal values of pH/MII parameters for children.

In conclusion, in the present study, a high proportion of SMID patients had relatively high SPLs, regardless of the presence of GERD, compared with healthy subjects. The SPL in SMID patients might be affected by several distinctive characteristic factors in addition to GER, and this condition might increase the risk of the other critical complications, such as aspiration pneumonia and tooth erosion, although further studies will be required to confirm these suspicions.

Conflict of interest

The authors have no conflicts of interest to disclose.

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