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Acrosomal vesicle protein in predicting progression of pancreatitis in Chinese patients with acute pancreatitis

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Abstract: The present preliminary investigation was designed to identify biomarkers in the progression of pancreatitis in Chinese patients with acute pancreatitis. Chinese patients aged <60 years with a confirmed diagnosis of acute pancreatitis were enrolled. A saliva sample was collected using salimetrics oral swab in precooled polypropylene tubes to prevent degradation of sensitive peptides. All samples were then centrifuged at 700 × g for 15 min at 4°C to remove debris. The supernatant of each sample was fractionated into 100µL aliquots and frozen at -70°C until analyses using affymetrix HG U133 Plus 2.0 array technique. Bedside index for severity acute pancreatitis (BISAP) score and CT severity index were recorded for each enrolled patient to assess the progression and severity of acute pancreatitis. Data from a total of 210 patients (105 patients in each group) were analyzed. Among identified biomarkers, acrosomal vesicle protein 1 was significantly higher in patients with disease progression as compared to patient without disease progression. Logistic regression model showed that acrosomal vesicle protein 1 (ACRV1) were positively correlated with the progression of diseases. The present reports showed that a mRNA salivary biomarker (ACRV1) are associated with progression of pancreatitis in patients with early stage of pancreatitis. This study suggest that mRNA salivary biomarker (ACRV1) is a predictor of pancreatitis progression.

Keywords: Acute pancreatitis, acrosomal vesicle protein 1, biomarkers.

INTRODUCTION

Acute pancreatitis (AP) is one of the most prevalent gastrointestinal disorders (Lotte, 2020). Based on the severity of AP, it is being classified as mild, moderate and severe as per the Atlanta Classification (Revised) system. Pancreatic inflammation was observed in all category of patients (Wadhwa, 2017; Popov, 2017); however, magnitude of inflammation varied based on the severity of AP. Severe cases of AP was characterized by damage of one or more organ system, with local and systemic complications (Baron, 2020). In comparison to mild case of AP, severe cases of AP have worst prognosis and require hospitalization or prolongation of hospitalization and high mortality rate (He, 2022; Baron, 2020; Kui, 2022) . Among patients of mild severity, 10 to 20% of patients progressed to severe form of AP, which can be fatal and difficult to manage at later stage. Hence, an early detection or prediction of disease progression is the key for effective management of AP (He, 2022; Kui, 2022). There are several biomarkers namely CRP, IL-6 and procalcitonin to predict the severity of AP during initial clinical phase (Chen, 2017; Komolafe, 2017; Bao, 2022; Kim, 2019).

Tumor necrosis factor-alpha (TNF-α) and Interleukin-1 (IL)-1 are playing key role in pathophysiology of AP and one of predicting factor/risk factor for developing severe cases of AP. Inflammatory biomarkers especially cytokines are the key biomarkers of disease severity at the time of hospitalization (He, 2022; Kui, 2022; Komolafe, 2017; Bao, 2022; Kim, 2019; Cho, 2018). However, cytokines are not cost effective and mostly not available in several small sized laboratories in rural area.

A simple and precise biomarker for early detection of disease progression (mild to severe cases of AP) are much needed. Also, an appropriate and cost-effective biomarker that predicts disease progression during initial course of AP is warranted. There are no reports of salivary biomarkers in AP.

This study evaluated the role of salivary biomarkers in AP and its role in progression of AP. There is no study evaluating the role of Salivary biomarkers in predicting progression of pancreatitis in Chinese patients with AP. Therefore, the present preliminary investigation was designed to identify salivary biomarkers in the progression of pancreatitis in Chinese patients with acute pancreatitis. Also, the present study is kind of pilot investigation to know whether progression of pancreatitis is associated with salivary mRNA biomarkers.

MATERIALS AND METHODS

Patients and ethics

Chinese patients aged <60 years with a confirmed diagnosis of acute pancreatitis were enrolled. Written informed consent was obtained from each enrolled patient. The study was initiated after obtaining ethical

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approval vide EC approval reference number BH/2021-IRB-03487/0345 from the Institutional Review Board of Budui Hospital. The procedures used in the study were in line with the ethical principles laid down in the Helsinki Declaration and its later amendments (Huh JH, 2018). Patients with a history of severe renal impairment, any type of cancer, liver disease, lung disease, severe coronary artery disease (CAD) and thyroid disease were excluded. Moreover, patients with any other pathology likely to affect the outcome of study, and patients who received concomitant and contra-indicated medications, as well as patients undergoing any other form of surgery, were excluded.

Study procedure

Each subject was asked to chew a gum base which neither contain fragrance nor flavoured ingredients. After 5 min of chewing gum, whole saliva was collected from each subject using salimetrics oral swab in precooled polypropylene tubes to prevent degradation of sensitive peptides. All samples were then centrifuged at 700 × g for 15 min at 4 °C to remove debris.

The supernatant (upper 2/3) of each sample was fractionated into 100 µL aliquots and frozen at −70 °C until analyses. Bedside index for severity acute pancreatitis (BISAP) score and CT severity index were also recorded for each enrolled patient to assess the progression and severity of acute pancreatitis. CT severity index and BISAP score were calculated to predict the prognosis of AP.

The assessment tools that were used in the present study to diagnose AP and its progression were: clinical examination, laboratory investigations (serum amylase, and serum lipase), imaging techniques (CT severity index) and BISAP score. BISAP and CT severity index score were measured from each subject.

Messenger RNA biomarkers were measured from saliva samples using affymetrix HG U133 Plus 2.0 array technique. RNA was extracted from the saliva sample using MagMax Viral RNA Isolation Kit. Extracted RNA was augmented using the RiboAmp RNA Amplification kit. Complementary DNA was transcribed using GeneChip reagents. Chip hybridization including scanning were performed using microarray technique.

In the present study, patients of AP were divided in two groups which include patients who experienced progression of pancreatitis (Group 1) and patients with no progression of pancreatitis (Group 2). Each subject was followed for 3 to 6 months. Messenger RNA biomarkers identified using microarray study were compared in patients of both the groups. Also, BISAP and CT severity index score were compared between both the groups and the results were analyzed. Correlation of mRNA biomarkers with progression of pancreatitis was assessed.

STATISTICAL ANALYSIS

Since the study was a preliminary study designed to identify salivary biomarkers in the progression of pancreatitis in Chinese patients with acute pancreatitis. Therefore, no formal calculation of sample size was performed as the present study was a pilot investigation to know whether progression of pancreatitis is associated with salivary mRNA biomarkers.

Quantitative data (normal data) were analyzed using unpaired t-test, whereas quantitative data (non-normal data) were analyzed using Mann Whitney test. Quantitative data are presented as mean ± SD, whereas categorical data are expressed as percentage/proportion of patients, and were analyzed using Fisher exact test/chi-squared test. Significant differences were assumed at $p < 0.05$. Statistical analysis of data was performed using Graph Pad statistical analysis software version 9.0.

Correlation of mRNA biomarkers with progression of pancreatitis was also assessed using regression model.

RESULTS

Out of the 220 patients screened, a total of 210 patients (105 patients in each group) were enrolled. In the present study, patients of AP were divided in two groups (105 patients in each) which include patients who experienced progression of pancreatitis and patients with no progression of pancreatitis. Overall, the demography and baseline characteristics in both the treatments group was generally similar (table 1). Majority of patients are female. The most common cause of AP among enrolled patients was alcohol induced followed by gall stone. Majority of enrolled patients were obese or overweight.

Table 1: Patient characteristics

Characteristic	Group 1 N=105	Group 2 N=105
Age, median	64	62
Female sex, %	57	59
Smoking, %	65	71
Body-mass index (obese), %	48	51
Body-mass index (overweight), %	21	18
Etiology of AP		
Alcohol induced, %	76	78
Gall stone induced, %	24	22
Drinking, %	100	98

Values are expressed as Median for numerical variable and % of patients for categorical variables. Group 1: Patients with progression; Group 2: Patients without progression

Percentage of patients of mRNA salivary biomarkers among patients with and without diseases progression is described in table 2. A total of 9 mRNA salivary biomarkers were detected in patients of both the groups.

Table 2: % of patients of mRNA salivary biomarkers

mRNA salivary biomarkers	Group 1N=105	Group 2N=105	P value
ACRV1	89	07	<0.05
DMXL2	84	81	>0.05
DPM1	87	80	>0.05
CDKL3	2	3	>0.05
MBD3L2	1.5	2	>0.05
TPT1	3	4	>0.05
DMD	4.3	5.2	>0.05
GLTSCR2	3.6	3.8	>0.05
STIM2	2.2	3.9	>0.05

Values expressed as % patients reported. Values of P value based on categorical variables were calculated using Chi-square test. Group 1: Patients with progression; Group 2: Patients without progression.

Table 3: Selected salivary biomarkers to predict progression of AP by logistic regression model

mRNA salivary biomarkers	AUC	Sensitivity	Specificity	P value	Positive predictive value	Negative predictive value	Likelihood ratio
ACRV1	0.96	0.94	0.98	<0.05	0.87	0.47	1.76

Values expressed as % of patients.

Table 4: BISAP Score in patients with AP

BISAP Score	Group 1N=105	Group 2N=105
2	2	0
3	12	0
4	14	1
5	49	0.5

Table 5: CT severity index score in patients with AP

CT severity index score	Group 1N=105	Group 2N=105
0-3	0	96
4-6	87	4
7-10	13	0

Values expressed as % of patients. Group 1: Patients with progression; Group 2: Patients without progression.

Among identified mRNA salivary biomarkers, only acrosomal vesicle protein 1 (ACRV1), was significantly higher in patients with disease progression as compared to patient without disease progression. This indicates the ACRV1, was the key salivary biomarkers in predicting the progression of diseases.

As noted in table 2, among 9 biomarkers identified, ACRV1 was the most prevalent among the patients who had diseases progression. Therefore, the correlation of this biomarker with the disease progression was tested by logistic regression model. The results of disease progression are presented in table 3. Logistic regression model showed that ACRV1 biomarkers was positively correlated with the progression of diseases. The magnitude of correlation was statistically significantly. The likelihood ratio was significantly higher for the ACRV1. Sensitivity and specificity were also higher for the ACRV1.

BISAP Score in patients with AP is presented in table 4. Among progression group, majority of patients had BISAP Score of 4 and 5. A total of 12% of patients with progression had BISAP Score of 3. Also, total of 2% of patients with progression had BISAP Score of 2. This indicates that the majority of patients had low risk of mortality even they had diseases progression.

CT severity index score in patients with AP is presented in table 5. Among progression group, majority of patients had CT severity index score of 4 to 6. A total of 13% of patients with progression had CT severity index score of 7 to 10. In patients without progression, majority of patients had CT severity index score of 0-3. A total of 4% of patients with progression had CT severity index score of 4 to 6. This indicates that the majority of patients in progression group had moderate severity of AP, whereas 13% of patients in progression group had severe grade of AP severity. In non-progression group, majority of

patients in progression group had mild severity of AP, whereas 4% of patients in progression group had severe grade of AP severity.

DISCUSSION

This is the first study designed to identify salivary biomarkers in the progression of pancreatitis in Chinese patients with acute pancreatitis. Correlation of mRNA biomarkers with progression of pancreatitis using logistic regression model was also assessed. Among patients of mild severity, 10 to 20% of patients progressed to severe form of AP, which can be fatal and difficult to manage at later stage. Hence, an early detection or prediction of disease progression is the key for effective management of AP. There are several biomarkers namely CRP, IL-6 and procalcitonin to predict the severity of AP during initial clinical phase.

In this present study, a total of 9 mRNA salivary biomarkers were detected. Among identified mRNA salivary biomarkers, ACRV1 was significantly higher in patients with disease progression as compared to patient without disease progression. This indicates the ACRV1 was the key mRNA salivary biomarkers in predicting the progression of diseases. The correlation of ACRV1 with the disease progression was tested by logistic regression model showed that it was positively correlated with the progression of diseases. The magnitude of correlation was statistically significant ($p<0.05$). Sensitivity and specificity acceptable for ACRV1. In previous reports, it has been reported that combination of these mRNA biomarkers (ACRV1, DMXL2 and DPM1) could differentiate pancreatic cancer patients from chronic pancreatitis and healthy individuals (Liu, 2022; Yamashita, 2016). In contrast to this finding, our study showed that only ACRV1 was also effective in predicting the progression of AP.

In this study, among progression group, majority of patients had BISAP score of 4 and 5. A total of 12% of patients with progression had BISAP score of 3. Also, total of 2% of patients with progression had BISAP Score of 2. This indicates that the majority of patients had low risk of mortality even they had diseases progression. CT severity index score in patients with AP is presented in table 5. Among progression group, majority of patients had CT severity index score of 4 to 6. A total of 13% of patients with progression had CT severity index score of 7 to 10. In patients without progression, majority of patients had CT severity index score of 0-3. A total of 4% of patients with progression had CT severity index score of 4 to 6. This indicates that the majority of patients in progression group had moderate severity of AP, whereas 13% of patients in progression group had severe grade of AP severity. In non-progression group, majority of patients in progression group had mild severity of AP, whereas 4% of patients in progression group had severe grade of AP severity.

Earlier reports suggested that TNF-α and IL-1 play a key role in pathophysiology of AP and one of predicting factor/risk factor for developing severe cases of AP (Cho, 2018; Huh, 2018; Kim, 2019; Yamashita, 2016; Bao, 2022; Komolafe, 2017). Inflammatory biomarkers especially cytokines are the key biomarkers of disease severity at the time of hospitalization (Cho, 2018; Huh, 2018; Kim, 2019). However, cytokines are not cost effective and mostly not available in several small sized laboratories in rural area. IL-1 and TNF-α are known to increase visibility of immature granulocytes present in in peripheral blood that improve the activity of bone marrow and considered as one of most promising prognostic biomarkers in AP (Cho, 2018; Huh, 2018; Kim, 2019; Yamashita, 2016; Bao, 2022; Komolafe, 2017). There are several biomarkers available to predict prognosis of AP. However, a simple and precise biomarker for early detection of disease progression (mild to severe cases of AP) are much needed. Also, an appropriate and cost-effective biomarker that predicts disease progression during initial course of AP is warranted.

The results of this study recommended to further explore use of mRNA salivary biomarker (ACRV1) in predicting the diseases progression in AP patients. Further cost-effectiveness of mRNA salivary biomarker as one of diagnostic test for AP. Also, clinical applicability of the mRNA salivary biomarkers should be explored. However, pilot clinical study followed by large clinical study with adequate sample size should be conducted to provide recommendation for clinical use. The results of this study will serve the basis of future clinical investigation and may give a valuable information to scientific community and researcher for clinical applicability of the mRNA salivary biomarkers in predicting progression of AP.

Limitations of the study

The findings of the present trial cannot to be generalized to the Chinese population since the study was conducted at a single study center in China. A large clinical trial with appropriate sample size is needed to confirm the present findings.

CONCLUSION

The present reports showed that a mRNA salivary biomarker (ACRV1) are associated with progression of pancreatitis in patients with early stage of pancreatitis. This is the first study suggest mRNA salivary biomarker (ACRV1) is a predictor of pancreatitis progression.

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