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ROLE OF MYELOPEROXIDASE AND MALONDIALDEHYDE IN PANCREATITIS

General Surgery

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ABSTRACT

Introduction The etiology and pathogenesis of acute pancreatitis are not completely clear. Acute pancreatitis (AP) is an inflammatory condition varying from a mild self-limiting to a severe systemic disease. Findings indicated that Myeloperoxidase (MPO) level increases in patients with AP and hence these indicators can be used as diagnostic factors to predict inflammation severity in AP.

Aim This study aims to validate the role of activation of MPO and Malondialdehyde (MDA) as a biomaker marker in patients with Pancreatitis in Indian subcontinent.

Method 50 Patients of Pancreatitis attending general surgery OPD and admitted to General Surgery department of SSKM Hospital ,Kolkata, West Bengal, India were taken. ELISA was done for MPO and MDA assay which are directly proportional to the color intensity of the test sample. Statistical Analysis was performed with help of Epi Info (TM) 3.5.3. EPI INFO is a trademark of the Centers for Disease Control and Prevention.

Result Level of MPO was decreased in acute pancreatitis compared to chronic pancreatitis and normal pancreatitis which was statistically significant. We also found that MDA was increased in chronic pancreatitis compared to acute pancreatitis and normal pancreatitis though it was not statistically significant.

Conclusion Plasma Malondialdehyde and Myeloperoxidase may be a useful additional marker of severity in the very early stages of acute pancreatitis.

KEYWORDS

MPO, MDA, Biomaker ,Pancreatitis

INTRODUCTION

Acute pancreatitis (AP) is a potentially life threatening disease with varying severity of presentation [1,2]. Nearly 60%–80% of all cases of AP in developed countries are attributable to either gallstone disease or alcohol abuse [3, 4].

Alcohol consumption has been increasing in the developing countries, such as China and India,[5] due to rapid urbanization and increased affluence.

Myeloperoxidase (MPO) has been implicated in promoting tissue damage in various inflammatory diseases. The highest level of MPO was noted at the first day in patients with severe AP. A decrease of MPO blood level occurred during the first three days in all patients with necrotizing pancreatitis. [6]

The etiology and pathogenesis of Acute pancreatitis are not completely clear. Findings indicated that MPO level increases in patients with AP. It was revealed that after treatment, there were significant reductions in biomarker levels. [7]

Clinical studies have confirmed that patients with diabetes had an elevated risk of acute pancreatitis Showed elevated serum amylase and lipase levels, increased myeloperoxidase (MPO) expressions in pancreatic and pulmonary tissues as well as increased apoptotic acinar cells after AP induction. [8] Acute pancreatitis (AP) is an inflammatory condition varying from a mild self-limiting to a severe systemic disease. Excessive recruitment of leukocytes is an important pathophysiological feature and myeloperoxidase (MPO) forms an important part of neutrophil induced inflammation. [9]

Acute pancreatitis (AP) is a common inflammatory disease mediated by damage to acinar cells and subsequent pancreatic inflammation with infiltration of leukocytes. [10]There is increasing evidence of the role of adipose tissue on the systemic effects of acute pancreatitis. [11]Intrapancreatic activation of digestive enzymes is a key event in the parenchymal cell injury of pancreatitis.[12] Release of oxygen free radicals is increased in acute pancreatitis, but whether this can be used to predict clinical severity is not known.Plasma malondialdehyde may be a helpful additional marker of severity in the very early stages of acute pancreatitis.[13]

The onset of complications is associated with high malondialdehyde concentration.[14]The mouse model of severe acute pancreatitis (SAP) could be induced with caerulein and LPS. [15]

The significantly increased plasma levels of MDA indicate that oxidative stress is present in patients with Chronic Pancreatitis (CP) and that it may play a role in initiation and maintenance of inflammation within the pancreatic tissue in CP patients.[16]The role of oxidative stress in the pathophysiology of acute pancreatitis and outcomes of antioxidant therapy as a therapeutic agent in the treatment of acute pancreatitis.[17]Study supported that hydrogen could be used as a novel treatment in chronic pancreatitis patient in the future.[18].

There are various biomarker markers in Pancreatitis with variable sensitivity and specificity. This study aims to validate the role of activation of MPO and MDA as a biomaker marker in patients with Pancreatitis in Indian subcontinent.

MATERIAL AND METHODS

50 Patients of Pancreatitis attending general surgery OPD and admitted to General Surgery department of SSKM Hospital, Kolkata, West Bengal, India were taken.

ASSAY PROCEDURE:

MPO :

For 0.5 M H_2SO_4 :-
0.7 ml Conc. H_2SO_4 + 24.3 ml TDW = 25 ml
2mM TMB : (Tetramethylbenzedine)
0.00096 g TMB + 2 ml DMF (N,N Dimethyl formamide)
0.3M H_2O_2 :-
0.536 ml H_2O_2 + 1.464 TDW (1ml)
10 ul sample + 50 ul buffer + 30 ul TMB + 10 ul H_2O_2 + 500 ul H_2SO_4

During stress condition the neutrophilic infection take place at that position. But when H_2O_2 is added,H_2O_2 breaks into water and oxygen radical. When TMB is added it gives blue color and on further addition of H_2SO_4 it gives yellow color. Reading is taken at 450.

1. All reagents and samples were brought to room temperature (18 - 25ºC) before use. It is recommended that all standards and samples be run at least in duplicate. 2. Removable 8-well strips were labeled as appropriate for experiment. 3.100 µl of each standard and sample were added into appropriate wells. Wells were covered and incubated for 2.5 hours at room temperature with gentle shaking. 4. The solution was discarded and washed 4 times with 1XWash Solution. Each well was washed by filling with Wash Buffer (300 µl) using a multi-channel Pipette or auto washer. Complete removal of liquid at each step was essential for good performance. After the last wash, any remaining Wash Buffer was removed by aspirating or decanting. The plate was inverted and blotted against clean paper towels. 5. 100 µl of 1X prepared biotinylated antibody was added to each well. Incubated for 1 hour at room temperature with gentle shaking. 6. The solution was discarded. Repeated the wash as in step 4. 7. 100 µl of prepared Streptavidin solution was added to each well. Incubated for 45 minutes at room temperature with gentle shaking. 8. The solution was discarded. Repeated the wash as in step 4. 9. 100 µl of TMB One-Step Substrate Reagent (Item H) was added to each well. Incubated for 30 minutes at room temperature in the dark with gentle shaking. 10. 50 µl of Stop Solution (Item I) was added to each well. Reading at 450 nm immediately

MDA protocol: Ray Biotech

Reagents:

1) 24% TCA
2) 0.67% TBA
3) n-butanol

Procedure:

1) 1ml serum sample+ 1ml of 24% TCA
2) Vortex and incubate at RT for 20mins
3) Centrifuge at 2000rpm for 20mins
4) Take 2ml of protein free supernatant
5) Add 1ml of 0.67% TBA and vortex.
6) Heated at 95 degree for 1 hr and sup was then cooled to RT
7) Pinkcoloured sup was extracted in 2ml n-butanol and vortex
8) Reading at 535nm

STATISTICALANALYSIS

Statistical Analysis was performed with help of Epi Info (TM) 3.5.3. EPI INFO is a trademark of the Centers for Disease Control and Prevention. test was used to test the association of different study variables. t-test was used to compare the means. Significance level was set at 0.05 and confidence intervals were at 95 percent level.

RESULT AND ANALYSIS

We found that 2(4.0%) patients were ≤20 years of age, 14(28.0%) patients were 21 to 30 years of age, 6(12.0%) patients were 31 to 40 years of age, 15(30.0%) patients were 41 to 50 years of age, 4(8.0%) patients were 51 to 60 years of age, 4(8.0%) patients were 61 to 70 years of age and 5(10.0%) patients were 71 to 80 years of age. It was found that 15(30.0%) patients were alcoholic, 3(6.0%) patients had ascites, 6(12.0%) patients had chronic pancreatitis, 24(48.0%) patients had gall stone and 2(4.0%) patients had osteoarthritis. We found that 20(40.0%) patients were female and 30(60.0%) patients were male.

It was found that in acute type, the mean MPO (mean±s.d.) of the patients was 1.4097 ± .4860 U/ml. In chronic type, the mean MPO (mean±s.d.) of the patients was 2.5667 ± .3086 U/ml. In normal type, the mean MPO (mean±s.d.) of the patients was 2.8250 ± .0500 U/ml. Distribution of mean MPO vs. type was statistically significant (p<0.0001). We found that in acute type, the mean MDA (mean±s.d.) of the patients was 2.4435 ± 1.0161 nM/ml. In chronic type, the mean MDA (mean±s.d.) of the patients was 2.7140 ± 1.2305 nM/ml. In normal type, the mean MDA (mean±s.d.) of the patients was 1.7625 ± .2901 nM/ml. Distribution of mean MDA vs. type was not statistically significant (p=0.2801).

We found that in female, the mean age (mean±s.d.) of the patients was 3.5000 ± 1.6059 years. In male, the mean age (mean±s.d.) of the patients was 3.9000 ± 1.7685 years. Distribution of mean age vs. sex was not statistically significant (p=0.4207). It was found that in female, the mean MPO (mean±s.d.) of the patients was 1.6700 ± .6554 U/ml. In male, the mean MPO (mean±s.d.) of the patients was 2.0033 ± .7527 U/ml. Distribution of mean MPO vs. sex was not statistically significant (p=0.1133). We found that in female, the mean MDA (mean±s.d.) of the patients was 2.8695 ± 1.1145 nM/ml. In male, the mean MDA (mean±s.d.) of the patients was 2.2040 ± .9546 nM/ml. Distribution of mean MDA vs. sex was statistically significant (p=0.0285).

We found that in alcoholic, the mean age (mean±s.d.) of the patients was 40.8000 ± 17.3255 years. In ascites, the mean age (mean±s.d.) of the patients was 57.3333 ± 28.0416 years. In chronic, the mean age (mean±s.d.) of the patients was 44.6667 ± 23.7795 years. In gall stone, the mean age (mean±s.d.) of the patients was 43.1667 ± 11.6158 years. In osteoarthritis, the mean age (mean±s.d.) of the patients was 41.0000 ± 14.1421 years. Distribution of mean age vs. sex was not statistically significant (p=0.6180). It was found that in alcoholic, the mean MPO (mean±s.d.) of the patients was 2.2400 ± .6367 U/ml. In ascites, the mean MPO (mean±s.d.) of the patients was 1.7333 ± .9292 U/ml. In chronic, the mean MPO (mean±s.d.) of the patients was 1.9000 ± .7589 U/ml. In gall stone, the mean MPO (mean±s.d.) of the patients was 1.6542 ± .6724 U/ml. In osteoarthritis, the mean MPO (mean±s.d.) of the patients was 1.8000 ± 1.4142 U/ml. Distribution of mean MPO vs. association was not statistically significant (p=0.1887). We found that in alcoholic, the mean MDA (mean±s.d.) of the patients was 2.3067 ± 1.0434 nM/ml. In ascites, the mean MDA (mean±s.d.) of the patients was 1.7367 ± .4488 nM/ml. In chronic, the mean MDA (mean±s.d.) of the patients was 2.1767 ± 1.0585 nM/ml. In gall stone, the mean MDA (mean±s.d.) of the patients was 2.6875 ± 1.0771 nM/ml. In osteoarthritis, the mean MDA (mean±s.d.) of the patients was 3.0700 ± 1.7395 nM/ml. Distribution of mean MDA vs. association was not statistically significant (p=0.4343).

Table: Distribution of age in years, association, sex and type

		Frequency	Percent
AgeinYears	**≤20**	2	4.0%
	21to30	14	28.0%
	31to40	6	12.0%
	41to50	15	30.0%
	51to60	4	8.0%
	61to70	4	8.0%
	71to80	5	10.0%
	Total	50	100.0%
Association	**Alcoholic**	15	30.0%
	Ascites	3	6.0%
	Chronic	6	12.0%
	Gallstone	24	48.0%
	Osteoarthritis	2	4.0%
	Total	50	100.0%
Sex	**Female**	20	40.0%
	Male	30	60.0%
	Total	50	100.0%
Type	**Acute**	31	62.0%
	Chronic	15	30.0%
	Normal	4	8.0%
	Total	50	100.0%

Table: Distribution of mean age, MPO U/ml, MDA nM/ml

	Number	Mean	SD	Minimum	Maximum	Median
Age	50	43.4000	16.0433	15.0000	77.0000	43.0000
MPO U/ml	50	1.8700	.7274	0.8000	2.9000	1.7500
MDAnM/ml	50	2.4702	1.0627	1.0800	4.6000	2.0150

DISCUSSION

It was found that 31(62.0%) patients had acute pancreatitis , 15(30.0%) patients had chronic pancreatitis and 4(8.0%) patients were normal. We found that the mean age (mean±s.d.) of the patients was 43.4000 ± 16.0433 years. It was found that the mean MPO (mean±s.d.) of the patients was 1.8700 ± .7274 U/ml. It was found that the mean MDA (mean±s.d.) of the patients was 2.4702 ± 1.0627 U/ml.

Chooklin S et al [6] found that the highest level of MPO was noted at the first day in patients with severe AP. A decrease of MPO blood level occurred during the first three days in all patients with necrotizing pancreatitis. The development of pancreatitis-associated lung injury and purulent complications was accompanied by increased MPO levels. MPO blood level is dependent on the severity of AP and on cytokine blood levels.

Present study found that level of MPO was decreased in acute

Table: Distribution of mean age, MPO U/ml, MDAnM/ml with type

		Number	Mean	SD	Minimum	Maximum	Median	p-value
Age	Acute	31	43.0323	15.7765	20.0000	77.0000	45.0000	0.7726
	Chronic	15	42.6667	17.3809	15.0000	75.0000	42.0000	
	Normal	4	49.0000	16.1038	35.0000	72.0000	44.5000	
MPOU/ml	Acute	31	1.4097	.4860	0.8000	2.6000	1.4000	<0.0001
	Chronic	15	2.5667	.3086	2.2000	2.9000	2.8000	
	Normal	4	2.8250	.0500	2.8000	2.9000	2.8000	
MDAnM/ml	Acute	31	2.4435	1.0161	1.0800	4.6000	2.0300	0.2801
	Chronic	15	2.7140	1.2305	1.2200	4.3000	2.0300	
	Normal	4	1.7625	.2901	1.4000	2.0300	1.8100	
MPOU/ml	Female	20	1.6700	.6554	0.8000	2.9000	1.5000	0.1133
	Male	30	2.0033	.7527	0.8000	2.9000	2.0000	
MDAnM/ml	Female	20	2.8695	1.1145	1.7300	4.6000	2.2200	0.0285
	Male	30	2.2040	.9546	1.0800	4.6000	1.9600	
Age	Alcoholic	15	40.8000	17.3255	15.0000	67.0000	42.0000	0.6180
	Ascites	3	57.3333	28.0416	25.0000	75.0000	72.0000	
	Chronic	6	44.6667	23.7795	25.0000	77.0000	34.0000	
	GallStone	24	43.1667	11.6158	28.0000	74.0000	44.5000	
	Osteoarthritis	2	41.0000	14.1421	31.0000	51.0000	41.0000	
MPOU/ml	Alcoholic	15	2.2400	.6367	1.0000	2.9000	2.4000	0.1887
	Ascites	3	1.7333	.9292	1.1000	2.8000	1.3000	
	Chronic	6	1.9000	.7589	0.8000	2.9000	1.8000	
	GallStone	24	1.6542	.6724	0.8000	2.8000	1.5000	
	Osteoarthritis	2	1.8000	1.4142	0.8000	2.8000	1.8000	
MDAnM/ml	Alcoholic	15	2.3067	1.0434	1.1300	4.6000	1.9900	0.4343
	Ascites	3	1.7367	.4488	1.2200	2.0300	1.9600	
	Chronic	6	2.1767	1.0585	1.0800	4.2000	1.9600	
	GallStone	24	2.6875	1.0771	1.4000	4.6000	2.1750	
	Osteoarthritis	2	3.0700	1.7395	1.8400	4.3000	3.0700	

pancreatitis compared to chronic pancreatitis and normal pancreatitis, which was statistically significant. The difference of mean MPO was statistically significant in three groups.

It was found that in alcoholic, the mean MPO (mean±s.d.) of the patients was 2.2400 ± .6367 U/ml. Distribution of mean MPO was higher in alcoholic patients.

Abu-Hilal M et al [19] found that levels of malondialdehyde were raised in acute pancreatitis patients and increased in patients with severe compared with mild acute pancreatitis; 12 hours after admission plasma malondialdehyde was 4.42+/-0.54 micromol/L and 2.95+/-0.24 micromol/L in severe and mild pancreatitis, respectively (mean+/-SEM; P=0.007). Plasma malondialdehyde greater than 2.75 micromol/L at 12 hours after admission had high overall accuracy for predicting severe acute pancreatitis. Plasma malondialdehyde may be a helpful additional marker of severity in the very early stages of acute pancreatitis.

We also found that MDA was increased in chronic pancreatitis compared to acute pancreatitis and normal pancreatitis though it was not statistically significant. It was also found that level of MDA was significantly lower in male compared to female. It was found that in osteoarthritis, the mean MDA (mean±s.d.) of the patients was 3.0700 ± 1.7395 nM/ml which was higher but mean MDA was not statistically significant (p=0.4343).

CONCLUSION

We found that Myeloperoxidase was low in Acute Pancreatitis which was statistically significant. Malondialdehyde was high in female compared to male which was statistically significant. It was found that MPO was higher in alcoholic patients though it was not statistically significant. MDA was higher in Osteoarthritis patients. The results of the present study showed the MPO and MDA blood level is dependent on the severity of AP and can be helpful in our clinical management.

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