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Research Article

Lymphocytes to Neutrophils Ratio, the Immunologic Link between Chronic Pancreatitis and Pancreatic Cancer; A Single Center Study

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Abstract

Aim: The study aims to establish a relationship between pancreatic cancer and chronic pancreatitis, using NLR as a potential risk factor.

Methodology: The clinical data, blood dyscrasias, and immunohistopathological results of 400 observations and 200 healthy patients were studied in Pancreatic Disease Institute of Wuhan Union hospital in China. The group consisted of 256 male and 144 female subjects, during the follow-up, the Chronic pancreatitis group were divided into two group (patients performed surgery and surgery not performed). The possible etiological factors assessed in this study were: history of pancreatitis, neutrophil-to-lymphocytes ratio (NLR).

Results: From history, 25 patients with chronic pancreatitis was investigated to have the malignancy transformation. Of this group, Six (6) have malignancy transformation in 84 post-operated patients, whereas 19 patients have malignancy transformation in 116 non-operated patients. Of these 19 patients 7, have performed surgery of because of malignancy transformation The incidence of pancreatic cancer was significantly lower in patients who had received surgery for chronic pancreatitis than in those who had not undergone surgery (hazard ratio estimated by Cox regression 0.63 [0.8 - 1.2], 95% CI (P = 0.02).

The risk for pancreatic cancer in chronic pancreatitis patients, with high neutrophil-to-lymphocyte ratio HR 3.2 (95% CI 2.6 - 3.8).

For Chronic pancreatitis progressing to Pancreatic cancer with high neutrophil-to-lymphocytes ratio the adjusted hazard ratio was found to be 3.4 at 95% confidence interval (CI) (2.1 - 4.7).

Further, the immunohistochemistry results, combined with medical history confirmed 25 cases of chronic pancreatitis progressing to pancreatic cancer.

Conclusion: Our study demonstrated that the neutrophil-to-lymphocyte ratio can not only be used for providing prognostic significance, but it also can be regarded as a risk factor in the progression from chronic pancreatitis to pancreatic cancer.

Keywords: *Lymphocytes; Neutrophils Immunologic Link; Pancreatic Cancer; Chronic Pancreatitis*

Introduction

Pancreatic carcinoma and Chronic pancreatitis represent the second and third leading causes of hospitalization requiring surgical intervention in Pancreatic Disease Institute of Union Hospital in Wuhan, China [1]. In the US, pancreatic cancer has been identified as the fourth cause of cancer-related deaths, while being 5th in European Union and 7th in China [2]. In the Western world, its incidence is currently 10/100,000 pa with rising morbidity among females. Chronic pancreatitis arises due to permanent fibrosis of pancreatic tissue [3]. In 20 - 30% of cases of chronic pancreatitis, mass lesions can be found in the pancreas. [3]. Pancreatitis is very likely to be a sequel of pancreatic carcinoma due to obstruction to the flow of pancreatic juices. Reports have shown 2% of patients with acute pancreatitis have concurrent pancreatic cancer [4-7]. Moreover, few studies have shown the strong association between medical history of pancreatitis in relation to pancreatic cancer [8-11]. The contribution of chronic inflammation in the pathogenesis of cancer is related to growth factor-influenced cellular proliferation [12,13]. This relationship strongly suggests the existence of a pathologic connection between pancreatitis and pancreatic cancer. In addition, there is evidence from studies that proves that inflammation is related to the development of cancer [14]. Virchow's hypothesis which came up during the 19th century was the first evidence to establishing this link, and in following years, epidemiological researches consolidated his hypothesis by ascertaining the etiological link between chronic pancreatitis and Pancreatic cancer [14].

From history, Patients with CP has been identified to have a higher incidence of pancreatic carcinoma [15,21] and some patients with history of hereditary pancreatitis can be suggested to have 40% of potential risk of developing pancreatic cancer. Similar inflammatory infiltrates and desmoplasia in Chronic pancreatitis (CP) and PDA tissues indicate coinciding inflammatory responses in the two diseases [15,21].

Pancreatic cancer is a disease which carries a poor prognosis and therefore, finding out the pathologic link between the 2 diseases is critical to reduce mortality rates by making earlier diagnoses. In the majority of cases, the diagnosis of pancreatic cancer can only be made at late stages, leaving only an extremely limited the window of opportunity for curative operation [15]. At advanced stage, resection is no longer the treatment modality of choice in more than 80% of PC patients due to extensive metastatic disease, resulting in a low five- year survival rate [16].

Inflammation needs to be maintained for a long period of time before it can induce metaplastic change in the cells. The influence of inflammatory mediators during the entire inflammatory phase lead to changes in gene expression and ultimately resulting in activation of neoplastic pathways. Eventually, systemic inflammations release a number of inhibitory immunologic mediators, most notably interleukin-10 (IL-10) and transforming growth factor-beta [18] leading to thrombocytosis and lymphocytopenia. However, T-lymphocytes, polymorph nuclear neutrophils (PMN) are found in chronically inflamed tissues and tumors as reviewed by di Carlo., *et al.* [19] and the derived neutrophil to lymphocyte ratio (dNLR) has therefore been proposed as an easily determinable prognostic factor in cancer patients.

The most recognized risk factors linked to development of pancreatic cancer in chronic pancreatitis patients are obesity, alcohol, cigarettes smoking, diabetes, gallbladder disease, ulcer, chronic pancreatitis and age [19]. Chronic pancreatitis and pancreatic cancer are two pathologies noticed to be similar anatomically and physiologically.

The polymorphism of the neutrophils and the disturbance of the immune system during the episodes of chronic pancreatitis and pancreatic cancer, are the reasons to present this work entitled as follows; Lymphocytes and Neutrophils the immunologic link between chronic pancreatitis and pancreatic cancer, a single center study.

Materials and Methods

Patients

It is a descriptive, preliminary and single center study; carried out on two groups (Case and control).

The patients chosen for case group were those who had chronic pancreatitis (n = 200) and pancreatic cancer (n = 200). The control group represents the healthy population (those patients do not have any history of Chronic Pancreatitis and Pancreatic Carcinoma and any other pathologies) (n = 200).

History

Patients' history was taken and they were arranged in 5-year age groups, to be analyzed for Cox regression multivariable analyses. Data regarding potential risk factors including neutrophil-to-lymphocytes ratio (NLR), the potential, cumulative malignancy transformation risk, cigarette smoking, alcohol consumption, diabetes mellitus, gallbladder disease and ulcer were collected in both groups (CP and PC).

During the follow-up, the patients in chronic pancreatitis group were divided in two groups:

1. Patients who underwent surgery and
2. Patients without surgical intervention

Tissue and blood collection in patients with chronic pancreatitis and pancreatic cancer

Tissue samples of pancreatic carcinoma patients, and chronic pancreatitis patients were collected from patients who had performed surgery for pancreatic carcinoma or chronic pancreatitis in the pancreatic disease institute of Wuhan union hospital of china. They were prepared for immunohistochemical studies by formalin and paraffin fixation and embedding. Control group has been identified in another department of Internal Medicine.

Investigations

CP and PC Patients were asked to have (1) blood routine examination results to investigate neutrophils-to-lymphocytes ratio (NLR), (2) histoimmunochemistry result, (3) tumors marker test (4) CT, MRI and (5) post-op cytopathological result. In this retrospective study, it were included patients who were diagnosed with chronic pancreatitis and with at least 5 years of follow-up.

Exclusion criteria

Patients with biliary diseases, small-cell carcinoma, non-differentiated adenocarcinoma, mucinous cyst adenocarcinoma, islet cell, or papillary cystic neoplasm, were excluded from the study.

Evaluation of the risk factors in control group

In our study, we had to evaluate whether the NLR ratio could be reviewed as a potential risk factor for pancreatic cancer in the control group.

Operational definition

Incidence

Incidence is the number of the new cases during one period of study.

Statistical analysis

The possible associations between pancreatic cancer status, demographic features and potential risk factors were assessed using Multivariate Cox regression adjusting for neutrophil-to-lymphocytes ratio, age, alcohol abuse, sex, history of chronic pancreatitis,

gallbladder disease, ulcer disease, smoking and the hazard ratios (HRs) with 95% confidence interval(CIs) were estimated directly from the reported data.

The statistical analysis was performed using Stata version 12.0 software (Stata Corporation, college Station, Texas, USA). Figures and cumulative risk factor were obtained using software graph pad Prismv5.01. P value smaller than 0.05 was regarded as statistically significant (CI = 95%).

Risk factor for Pancreatic cancer	Chronic Pancreatitis Group	Hazard Ratio	Multivariate Cox regression analysis P value
NLR	6.74		
Neutrophil	7.08	3.2 [2.6 - 3.8]	0.0013
Lymphocytes	1.05		
Gender			
Men	123	1.5 [1.2 - 2.2]	0.4751
Women	77		
History of pancreatitis duration			
< 5	2		
10	3		
15	4	4.7 [3.8 - 5.6]	0.0015
20	4		
25	5		
> 25	7		
Diabetes			
Yes	47	3.1 [2.3 - 3.8]	0.026
No	153		
Smoking			
Yes	78	3.04 [2.8 - 3.6]	0.018
No	122		
Alcohol drinking			
Yes	66	1.8 [1.6 - 2.4]	0.022
No	134		
Gall bladder disease			
Yes	15	1.42 [1.14 - 2.05]	0.0241
No	185		

Table 1: *Distribution of patient according to the demographic and risk factor's analysis.*

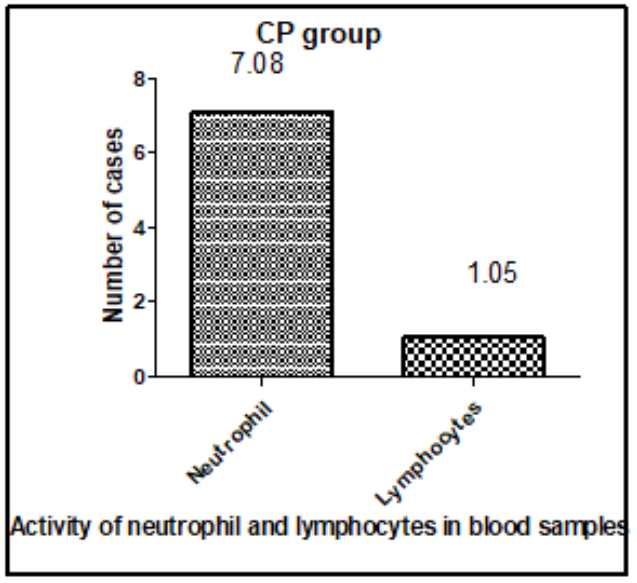

Figure A: *Chronic pancreatitis group, in this figure Neutrophil to lymphocytes ratio is equal 6.74 (NLR = 6.74).*

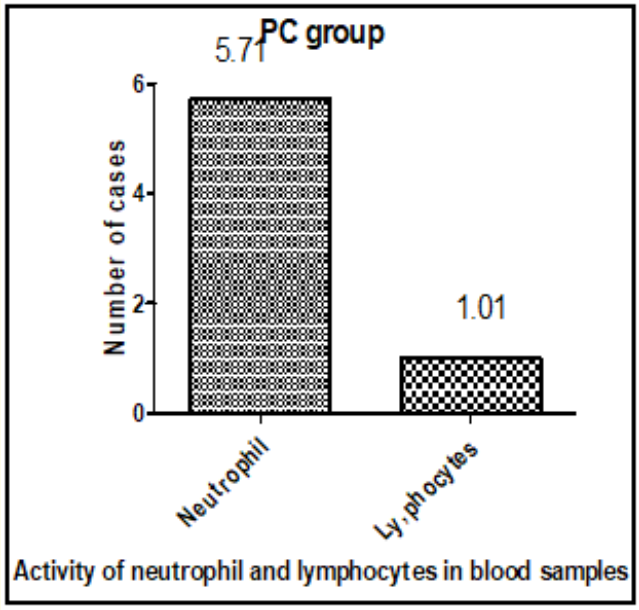

Figure B: *Pancreatic cancer group, the neutrophil to lymphocytes is equal at 5.65 (NLR = 5.65).*

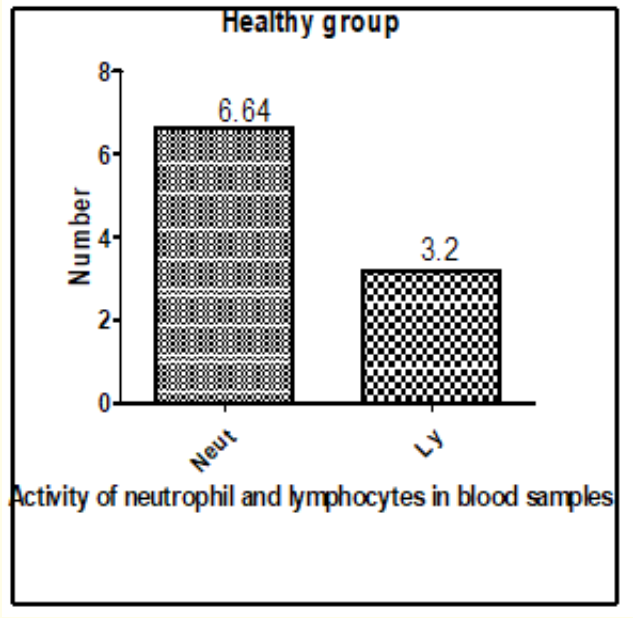

Figure C: *Healthy group, neutrophil to lymphocytes ratio (NLR = 2.07).*

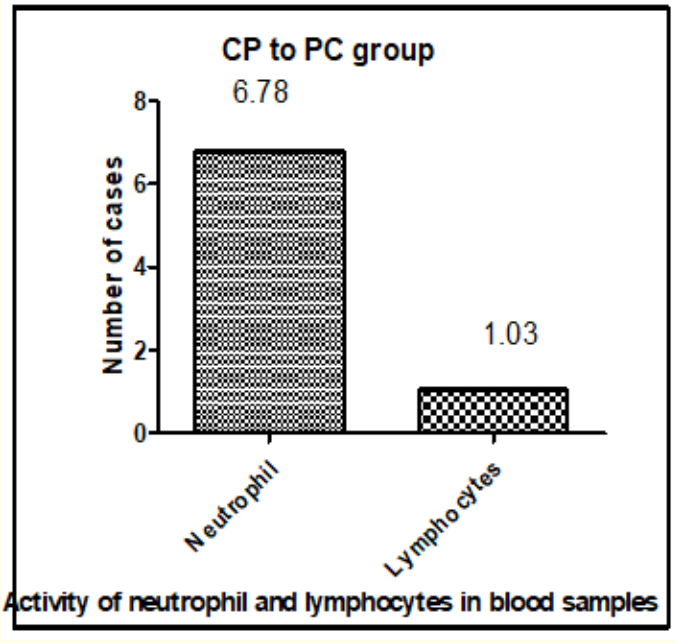

Figure D: *Chronic Pancreatitis progressed to the PC (NLR = 5.58).*

Risk factor analysis for malignancy transformation in NLR	Chronic Pancreatitis Progressing to Pancreatic Cancer N = 25	Hazard Ratio 95% CI	P value Multivariate analysis
NLR	5.58		
Neutrophil increasing			
Yes	10		
No	15		
Lymphocytes decreasing			
Yes	8	3.6 [2.7 - 4.9]	0.0026
no	17		

Table 2: *Risk factor's analysis according NLR in CP to PC group.*

Neutrophil range (2-7 G/L) the mean of neutrophil is 4,5G/L.

Lymphocytes range (0.8-4G/L) the mean of lymphocytes is 2,4G/L.

Surgical and no surgical act	Chronic Pancreatitis Group n = 200	Malignancy transformation	Multivariate Cox régression analysis for incidence
Performed Surgery	84	6 (7.1%)	0.63 [0.8 - 1.2]
Surgery not Performed	116	19 (16.3%)	P = 0.02
			2.11 [1.7 - 3.2]

Table 3: *Distribution of Chronic pancreatitis patients according to the incidence (surgical act and the malignancy transformation).*

Of the 19 patients 7, have performed operation of because of malignancy transformation.

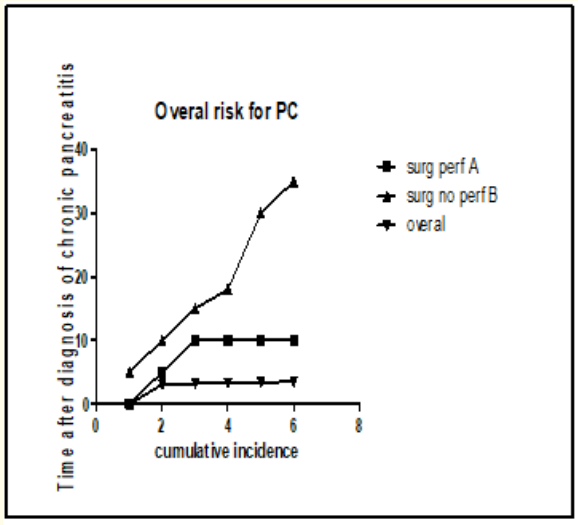

Figure E: *Number of patients at risk (Events).*

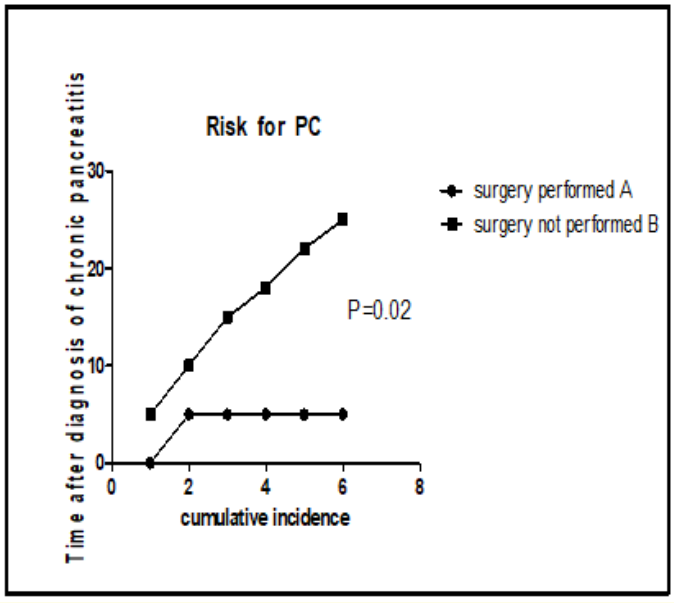

Figure F: *Number of patients at risk (Events).*
200(11) 160(6) 93(3) 51(2) 32(2) 25(1) 15(0) 7(0) 1(0)
Surgery not performed 114 (6) 95(5) 48(4) 25(3) 7(1) 1(0)
Surgery performed 86(0) 58(2) 36(2) 15(2) 10(0) 1(0)

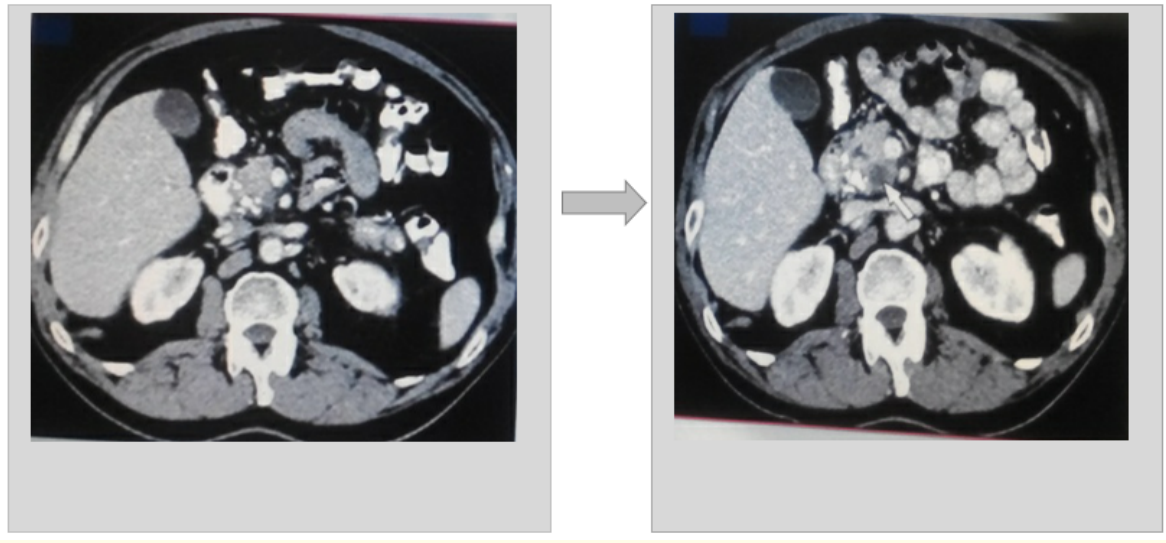

Figure G and H: *Showed us the malignancy transformation cases after several years follow-up among patient in the same center.*

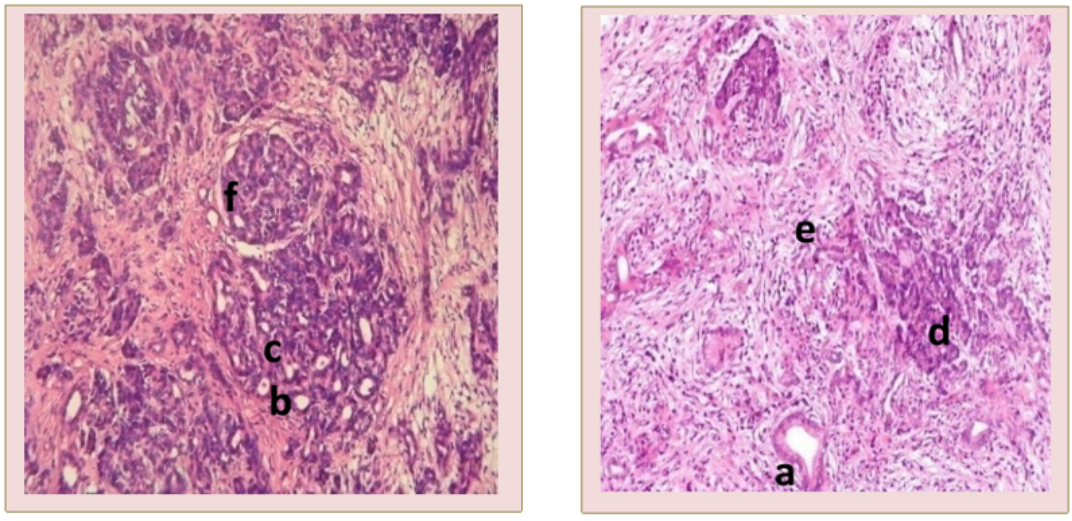

Figure I and J: *Immunohistochemistry results of CP and PC. a- (cancer cell); b- (calcifications); c-d (neutrophil with nucleus); e-f (lymphocytes).*

In these histological examination we can found clusters of calcified bodies in figure I and J, cancer cells could be recognized by the presence of granulocytic cytoplasm, vesicular nuclei with prominent neutrophilic nuclei and distinct borders..

Furthermore, the histo-immunochemistry analyzes, allowed us to clearly identify 25 cases of chronic pancreatitis which was transformed into pancreatic cancer, including 2 cases of low differentiation adenocarcinoma and 23 cases of adenocarcinoma.

Results and Discussion

From January 2009 to October 9th, 2014, 1441 patients were hospitalized in the pancreatic disease institute of Wuhan Union hospital, among 400 whom patients were eligible for our study (200 patients in CP group and 200 patients in PC group).

In the United States of America, two studies were carried out with a total of 1664 patients [21] to find the possible link between pancreatitis and risk for pancreatic cancer. 552 cases were studied in San Francisco Bay from 1991 to 1995, followed by 1131 cases in the MD Anderson cancer center group between 2001 and 2006.

In United Kingdom, a study on 1903 patient was carried out on the impact of the duration of diabetes mellitus and chronic pancreatitis to demonstrate the association between type 2 diabetes and pancreatic cancer risk [22].

In Taiwan over the 3 years period from 2000 to 2003, another study was carried-on 449,685 cases to investigate, the association between diabetes mellitus and pancreatic duct carcinoma [23].

However, the American study was a multicentric study, whereas ours is a single center study carried out at the pancreatic disease institute of Wuhan Union hospital of China.

According Wang., *et al.* the number of recruited patients varied from one country to another and according to studies subjects [1]. Indeed, the heterogeneous finding of this distribution were also documented in several recent medical literatures as they included participants of Asians [23], Americans [21], or European [22] origins.

A total of 25 cases (12, 5%) reported a history of pancreatitis which evolved into pancreatic cancer after 5 years follow-up (Table 1 and Figure E, F).

The overall incidence of chronic pancreatitis during 5 years of study from January 2009 to October 2014 was 2.7% out of 200 patients, with adjusted hazard ratio 4.7 95% confidence interval (CI) 1.1 - 5.6 (Table 2).

Our incidence are found to be inferior to the American, European and Japanese, study, but superior to the Taiwan study [21-24].

In fact, the potential cumulative malignancy transformation risk of 5, 10, 15, 20, 25 years is 2.1%, 5.4%, 7.3%, 11.2%, 15,1% respectively.

Of these 200 Chronic pancreatitis patients 84 have underwent surgery and 116 without surgical intervention.

Of our investigation we found that, Six (6) patients has been identified to have a malignancy transformation in 84 post- operated patients (Table 2, Figure F).

Furthermore, nineteen (19) patients has been investigate to have a malignancy transformation in 116 non-operated patients (Table 2). However, of these 19 patients, 7 have performed operation of because of malignancy transformation. The incidence of pancreatic cancer was significantly lower in patients who had received surgery for chronic pancreatitis than in those who had not undergone surgery ($p = 0.02$) (Table 2). It is evident that, the chronic pancreatitis is suggested to be one of the risk factors for the development of pancreatic cancer.

In light of our observation and according to the figure B, it is established that, the surgery for chronic pancreatitis has been found to decrease the risk for malignancy transformation.

Neutrophil-to-lymphocytes ratio (NLR)

In our study, the neutrophil -to-lymphocytes ratio was higher in chronic pancreatitis group compared to the pancreatic cancer group and healthy control group (Figure A-C), this increasing of NLR in CP group is a risk for CP patient to progressing into figure pancreatic cancer.

Further, the risk calculated for Chronic pancreatitis patients with increasing neutrophil-to-lymphocytes ratio was 3.2, at 95% confidence interval (CI) 2.6 - 3.8 (Table 2).

Consequently, we were able to conclude that these patients with the increased NLR were 3.2 times more likely to acquire pancreatic cancer.

Analysis of immune-histopathological results

Histological examination reveals clusters of calcified bodies in figure A and B, cancer cells can been identified by the presence of granulocytic cytoplasm, vesicular nuclei with prominent neutrophilic nuclei and distinct borders.

Immune-histochemical analysis revealed, focal immunoreactivity with reactive hyperplasia, metaplasia, and lymph node by region, and a strong nuclear positivity was observed in some chronic pancreatitis results. However, during chronic pancreatitis, lymphocytes and mononuclear cells have been identity to infiltrate the pancreas and contribute often to the local progression of the disease through T-lymphocyte mediated cytotoxicity and production of cytokines [28-31].

Hyperplasia is defined as an increase in number of cells, however, in metaplasia, the epithelium is normal in appearance but in an abnormal location.

Basically, hyperplasia is the first phase of the inflammatory process. Twenty-five cases with history of pancreatitis along with abundance of lymph node with multiple metaplasia have developed into pancreatic cancer.

Conclusion

According to our results a relationship between our immune systems and pancreatic disease (PC and CP) can be suggested. The neutrophil-to-lymphocyte ratio (NLR) can not only be used for providing prognostic significance, but also as a risk factor for assessing, the progress from chronic pancreatitis to pancreatic cancer. However, a history of pancreatitis must first be considered in conjunction with antecedent medical history and associated risk factors. In the light of the evidence put forward by our studies, pancreatitis may be viewed as an early clinical sign of pancreatic cancer in a number of patients. Therefore, it would be essential to consider pancreatic cancer within the differential diagnoses for pancreatitis. However, it is established that, the surgery for chronic pancreatitis has been found to decreases the malignancy transformation among patient in chronic pancreatitis.

Bibliography

1. Camara SN., *et al*. "Etiology, pathology, management and prognosis of chronic pancreatitis in Chinese population: A retrospective study". *Journal of Huazhong University of Science and Technology Medical Sciences* 35.3 (2015): 384-389.
2. Jemal A., *et al*. "Cancer statistics". *CA: A Cancer Journal for Clinicians* 60.5 (2010): 277-300.
3. Koninger J., *et al*. "Duodenum preserving pancreatic head resection in the treatment of chronic pancreatitis". *Roczniki Akademii Medycznej w Bialymstoku* 49 (2004): 53-60.
4. Balkwill F and Mantovani A. "Inflammation and cancer: back to Virchow?" *The Lancet* 357.9255 (2001): 539-545.
5. Pinho AV., *et al*. "Chronic pancreatitis: a path to pancreatic cancer". *Cancer Letters* 345.2 (2014): 203-209.
6. Tuveson DA and Neoptolemos JP. "Understanding metastasis in pancreatic cancer: a call for new clinical approaches". *Cell* 148.1-2 (2012): 21-23.
7. Mitchell P Fink. "Partial list of physiologic effects induced by infusing interleukin1 or tumor necrosis factor into human subjects". Sabist on textbook of surgery 19th edition.
8. Raimondi S., *et al*. "Pancreatic cancer in chronic pancreatitis aetiology, incidence, and early detection". *Best Practice and Research: Clinical Gastroenterology* 24.3 (2010): 349-358.
9. Szasz G. "A kinetic photometric method for serum γ-glutamyl transpeptidase". *Clinical Chemistry* 15.2 (1969): 124-136.
10. Lum G and Gambino SR. "Serum gamma-glutamyl transpeptidase activity as an indicator of disease of liver, pancreas, or bone". *Clinical Chemistry* 18.4 (1972): 358-362.
11. Beatty GL., *et al*. "CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans". *Science* 331.6024 (2011): 1612-1616.
12. Bedi MMS., *et al*. "CA 19-9 to differentiate benign and malignant masses in chronic pancreatitis: is there any benefit?" *Indian Journal of Gastroenterology* 28.1 (2009): 24-27.
13. Campbell AS., *et al*. "Macrophage inflammatory protein-3α promotes pancreatic cancer cell invasion". *Journal of Surgery Research* 123.1 (2005): 96-101.
14. Mazzieri R., *et al*. "Targeting the ANG2/TIE2 axis inhibits tumor growth and metastasis by impairing angiogenesis and disabling rebounds of proangiogenic myeloid cells". *Cancer Cell* 19.4 (2011): 512-526.

15. Mitchem JB., *et al*. "Targeting tumor-infiltrating macrophages decreases tumor-initiating cells, relieves immunosuppression, and improves chemotherapeutic responses". *Cancer Research* 73 (2013): 1128-1141.

16. Kaiser MH and Ellenberg SS. "Pancreatic cancer: adjuvant combined radiation and chemotherapy following curative resection". *Archives of Surgery* 120.8 (1985): 899-903.

17. Niederau C and Grendell JH. "Diagnosis of pancreatic carcinoma: imaging techniques and tumor markers". *Pancreas* 7.1 (1992): 66-86.

18. Yeo TP and Lowenfels AB. "Demographics and epidemiology of pancreatic cancer". *Cancer Journal* 18.6 (2012): 477-484.

19. Bosetti C., *et al*. "Pancreatic cancer: overview of descriptive epidemiology". *Molecular Carcinogenesis* 51.1 (2012): 3-13.

20. Lowenfels AB., *et al*. "Gallstone growth, size, and risk of gallbladder cancer: an interracial study". *International Journal of Epidemiology* 18.1 (1989): 50-54.

21. Andren-Sandberg A., *et al*. "Etiologic links between chronic pancreatitis and pancreatic cancer". *Scandinavian Journal of Gastroenterology* 32.2 (1997): 97-103.

22. Albert B., *et al*. "Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group". *The New England Journal of Medicine* 328.20 (1993): 1433-1437.

23. Ardengh JC., *et al*. "Endoscopic ultrasound and fine needle aspiration in chronic pancreatitis: differential diagnosis between pseudotumoral masses and pancreatic cancer". *Journal of the Pancreas* 8.4 (2007): 413-421.

24. Rickels MR., *et al*. "Detection, evaluation and treatment of diabetes mellitus in chronic pancreatitis: recommendations from Pancreas Fest 2012". *Pancreatology* 13.4 (2013): 336-342.

25. Zhang C., *et al*. "The early diagnosis of pancreatic cancer and diabetes: what's the relationship?" *Journal of Gastrointestinal Oncology* 5.6 (2014): 481-488.

26. Falconi M., *et al*. "Pancreatic head mass, how can we treat it? Chronic pancreatitis: surgical treatment". *Journal of the Pancreas* 1.3 (2000): 154-161.

27. Carr-Locke DL and Davies TJ. "Pancreatic juice gamma-glutamyl transferase, alanine transaminase, and alkaline phosphatase in pancreatic disease". *Digestive Diseases and Sciences* 25.5 (1980): 374-378.

28. Wilson Denise D. Manual of Laboratory and Diagnostic Tests (2008).

29. Lévy P., *et al*. "Epidemiology of chronic pancreatitis: burden of the disease and consequences". *United European Gastroenterology Journal* 2.5 (2014): 345-354.

30. Smailyte G., *et al*. "Educational differences in incidence of cancer in Lithuania, 2001-2009: evidence from census-linked cancer registry data". *European Journal of Cancer Prevention* 24.3 (2015): 261-266.

31. Siegel R., *et al*. "Cancer statistics, 2014". *CA: A Cancer Journal for Clinicians* 64.1 (2014): 9-29.

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