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TO STUDY *CHD-5* GENE POLYMORPHISM (4615T>C) IN PANCREATIC ADENOCARCINOMA PATIENTS AND PANCREATIC CANCER CELL LINES

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Conflicts of Interest: Nil
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Abstract:

BACKGROUND: Chromodomain helicase DNA binding protein-5 gene (*CHD-5*) has been identified as tumor suppressor gene. Human chromosome 1p36 is a region consistently deleted in cancers. *CHD-5* is located in this region. CHD-5 is the fifth member of a family of chromatin remodeling proteins. *CHD-5* is preferentially expressed in the nervous system and testis. *CHD-5* was first identified because of its location in a region of frequent deletion in neuroblastomas. Downregulation of *CHD-5* gene expression has been observed in neuroblastoma, breast cancer, colon cancer and ovarian cancer etc. This may be explained by deletions or other mutations.

Pancreatic cancer is the 4th leading cause of cancer death. The most common type is pancreatic adenocarcinoma (PA), comprises about 90% of all the malignant neoplasms of pancreas. Chromodomain helicase DNA binding protein-5 gene (*CHD-5*) has been identified as tumor suppressor gene. Downregulation of *CHD-5* gene expression has been observed in neuroblastoma, breast cancer, colon cancer and ovarian cancer etc. This may be explained by deletions or other mutations. A tumor-specific function-altering mutation is a well-established indicator for tumor suppressor genes in breast cancer and ovarian cancer etc. according to previous studies, but very less data is available about *CHD-5* gene in pancreatic cancer. Here, *CHD-5* gene polymorphism (4615T>C) has been studied in pancreatic adenocarcinoma and pancreatic cancer cell lines.

AIMS AND OBJECTIVES: To study *CHD-5* gene polymorphism (4615 T>C) in histopathologically/ cytologically confirmed cases of PA and pancreatic cancer cell lines.

MATERIALS AND METHODS: This study was conducted in the Department of Biochemistry of MAMC, New Delhi. The blood sample of 20 patients of PA were taken along with 20 healthy controls. We evaluated *CHD-5* gene polymorphism by using ASO-PCR after cell-free DNA extraction from plasma. We also did similar study in two pancreatic cancer cell lines: PANC-1 and MiaPaCa-2, after DNA extraction from cell lines followed by *CHD-5* gene polymorphism by using ASO-PCR.

RESULTS AND CONCLUSION: SNP 4615T>C (Ser1539Pro) was present in 85% of cases as compared to 75% in controls. However, the difference was not significant. So, there was no association between SNP 4615T>C in *CHD-5* gene and PA. SNP was present in PANC-1 but heterozygosity was seen in MIA PaCa-2 cell line.

Keywords: *CHD-5* gene, Pancreatic adenocarcinoma, Single nucleotide Polymorphism

Introduction

Pancreatic cancer is the 4th leading cause of cancer death and the most common type of pancreatic cancer is pancreatic adenocarcinoma (PA). It comprises about 90% of all malignant pancreatic neoplasms. It is one of the most lethal malignancies having poor prognosis with 5-year survival rate of approximately 5–7%1,2 and occurs mostly after the age of 45 years 3,4. It develops in a relatively symptom-free manner and is usually advanced at the time of diagnosis. Many new genes are identified to be involved in the development of cancer. A large number of genetic alterations have been identified. Promoter hypermethylation can lead to silencing of tumor suppressor gene (TSG) and the study of such alterations can be used as a screening tool in cancers5. Human chromosome 1p36 is a region consistently deleted in cancers6. Chromodomain helicase DNA-binding protein-5 (CHD-5) gene is

located in this region. CHD-5 protein is a member of a family of chromodomain enzymes that belong to the ATP-dependent chromatin remodeling protein superfamily. It is a member of a subclass of the chromatin remodeling SWI/SNF (SWItch/Sucrose Non-Fermentable) proteins. SWI/SNF chromatin remodelers are large, conserved complexes7. Proteins within this subclass contain a SWI-SNF-like helicase and two chromodomain motifs. These help in chromatin remodeling and affect gene transcription. Genes encoding subunits of SWI/SNF chromatin remodeling complexes are collectively altered in over 20% of human malignancies8. Recently, CHD-5 is identified as a TSG and the evidence has come principally from studies of neuroblastoma. CHD-5 expression is consistently down-regulated in primary neuroblastomas and cell lines. CHD-5 is regulated by DNA methylation of its promoter and histone modifications. The ability of CHD-5 to bind unmodified histone 3 is essential for tumor suppression9. CHD-5 is epigenetically silenced in neuroblastoma, colorectal cancer, breast cancer, cervical cancer, hepatocellular carcinoma, gastric cancer and lung cancer. Mutations in CHD-5 have been found in head and neck squamous cell carcinoma, prostate cancer, ovarian cancer, hepatocellular carcinoma, breast and colorectal cancer10.Loss of CHD-5 enhanced tumor proliferation whereas restoration of CHD-5 inhibited proliferation. The function of CHD-5 has mainly been studied in neural tissues where it was determined to control cell death and replication via the p19(ARF)/p53 pathway11. The study of specific cellular and molecular mechanisms of PA development and progression can be helpful to identify early detection strategies, preventative measures, and effective interventions.

Recent molecular genetic studies of a variety of tumor types have identified tumors with highly complex chromosome rearrangements and have found that relatively small regions of allelic imbalance and copy number gain may be quite common, for example, single nucleotide polymorphisms12,13. The presence of decreasingly sized aberrations being detected implies a continuum of genetic instability phenotypes from simple copy number changes affecting whole chromosomes and rearranged chromosomes14,15.

MATERIALS AND METHODS

This study was conducted in the Department of Biochemistry of Maulana Azad Medical College, New Delhi in conjunction with the Department of Gastrointestinal surgery and Department of Pathology of G. B. Pant Institute of Postgraduate Medical Education & Research, New Delhi. It was approved by the Institutional Ethical Committee, Maulana Azad Medical College.

STUDY DESIGN-

1. HOSPITAL BASED STUDY - Case control study

It was a pilot study and a sample size of convenience was taken. Twenty pancreatic adenocarcinoma patients who were admitted in Gastrointestinal surgery ward, GIPMER, New Delhi, for workup during the period from November 2015 to January 2017 were recruited for this study. The patients were recruited irrespective of stage of the disease. Twenty healthy persons of related age group were also recruited as controls in this study. The written informed consent was taken from cases and controls. A detailed history about the onset of disease and the treatment was taken. Staging was carried out as per the American Joint Committee on Cancer (AJCC) recommendations. Inclusion criteria for cases was histopathologically/cytologically confirmed cases of pancreatic adenocarcinoma and exclusion criteria was concurrent cancer of any other site. Inclusion criteria for controls was normal healthy volunteers of similar age group and exclusion criteria was prior history of malignancy or history of chronic disease or family history of pancreatic cancer (in first degree relatives). Blood sample was withdrawn under aseptic conditions. 3 ml blood was collected in EDTA vial for DNA and RNA work. Plasma was separated and stored in eppendorfs at -80oC till further analysis.

DNA EXTRACTION FROM PLASMA

Cell free DNA was isolated from plasma/serum DNA isolation Kit obtained from Epigentek Group Inc. Agarose gel electrophoresis was done and it was measured spectrophotometrically using nano drop (ND- 1000 from Nanodrop Technologies Inc) to confirm the presence of DNA.

2. IN-VITRO STUDY - Observational study

Pancreatic cancer cell-lines: MIA PaCa-2 and PANC-1, were procured from National Centre For Cell Science, Pune, India. Cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). It was grown in tissue culture flasks and plates.

DNA EXTRACTION FROM CELL LINES

DNA from monolayer cells was extracted using Genomic DNA Mini Kit (Geneaid Biotec Ltd., Taiwan) according to the manufacturer's instructions and was stored at -20°C.

ALLELE SPECIFIC (AS) PCR FOR CHD-5 4615 T>C

A 2 tube reaction was done to detect the presence or absence of each allele. 2 parallel reactions were carried out in separate tubes, one for the wild and the other for the mutant allele. Wild type specific and mutant specific forward primers were used separately in each tube. Common reverse primer was used in both the tubes. The primers were designed using the software Primer 3.149 Each reaction was performed in a total volume of 25 µL.

Primers: 4615 T>C

a. Wild forward: 5' GCCCGAGGGGAAGAAGC 3'- (25pmol/µL)
b. Mutant forward: 5' GGCCCGAGGGGAAGAAGT 3'- (25pmol/µL)
c. Common reverse: 5' AACAGAGGCTTTGGGGGAAG 3'- (25pmol/µL)

Annealing at 57oC (4715 T>C) for 45 seconds.

The amplified products of plasma DNA were resolved using electrophoresis in 2% agarose gel (mixed with Ethidium Bromide). The presence of a 224 bp long product confirmed the presence of 4615 T>C in the corresponding tube. Similarly, the amplified products of cell lines (MIA PaCa-2 and PANC-1) were also resolved.

All statistical analysis will be performed using Statistical Package for the Social Sciences (SPSS) software. The $p<0.05$ will be taken as statistically significant.

RESULTS

The mean age among study population was 50.52 years with standard deviation of 9.855 years. Minimum age was 27 years and maximum 70 years. It was seen that most patients of pancreatic adenocarcinoma were in the 50-59 years age group. No significant difference found in age or sex among cases and controls. There were twelve patients in Stage II, six patients in Stage I and two patients in Stage III. There was no patient in Stage IV of the disease in our study.

SINGLE NUCLEOTIDE POLYMORPHISM IN CHD-5 GENE - We studied SNP 4615T>C (Ser1539Pro) in CHD-5 gene in plasma DNA of pancreatic adenocarcinoma patients and controls using AS-PCR. Here, CC was mutant genotype. SNP 4615T>C was present in 85% of cases as compared to 75% in controls (Table 1, Figure 1). However, the difference was not significant. So, there was no association between SNP 4615T>C in CHD-5 gene and PA. SNP was present in PANC-1 but heterozygosity was seen in MIA PaCa-2 cell line (Figure 2).

Table 1: Distribution of CC,CT,TT genotypes at *CHD-5* (4615T>C) in cases and controls

SNP 4615T>C	Number of cases	Number of controls
CC(Mutant)	3	5
CT(Heterozygous)	17	13
TT(Wild)	0	2
Total	20	20

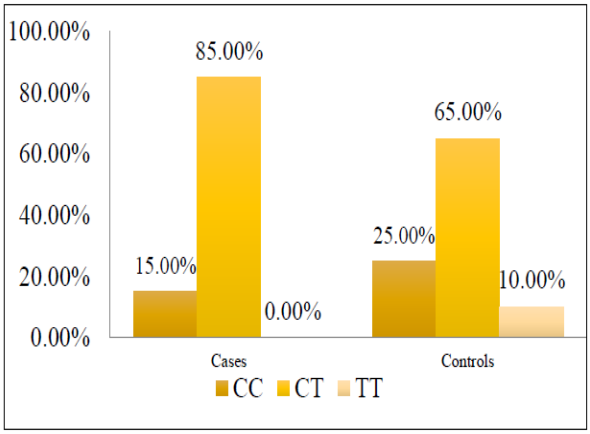

Figure 1: Bar diagram showing relationship between CHD-5 gene polymorphism (4615T>C) and pancreatic adenocarcinoma. (P-value is 0.22, statistically insignificant)

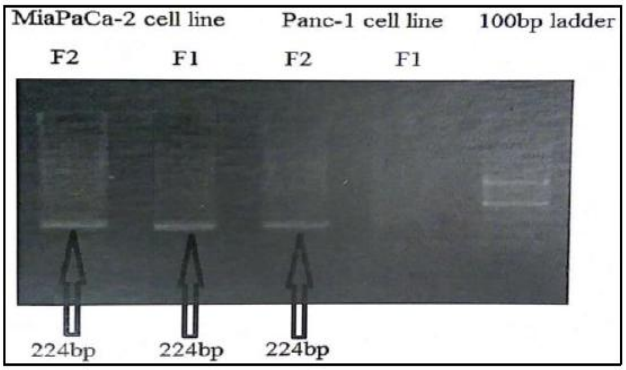

Figure 2: Agarose Gel Electrophoresis. Representative result showing polymorphism status of CHD-5 gene at 4615 in MIAPaCa-2 cell line showing genotype CT

(wild as well mutant) and PANC-1 cell line showing mutant genotype CC

DISCUSSION

Pancreatic cancer is the leading cause of cancer death and has become a global health priority16.More than 90% of pancreatic cancers are ductal adenocarcinomas, with neuroendocrine tumors constituting about 5%. In this study, we evaluated the promoter hypermethylation and gene expression of tumor suppressor gene CHD-5 in plasma DNA/RNA of pancreatic adenocarcinoma patients. Worldwide pancreatic cancer show highest incidence in the 60-80 years age group17. In our study, a majority of twenty patients were in the 50-59 years age group.

We studied SNP 4615T>C in CHD-5 gene in plasma DNA of pancreatic adenocarcinoma patients and controls. The similar study was done in pancreatic cancer cell lines. A tumor-specific function-altering mutation is a well established indicator for tumor suppressor genes. Sjoblom et al identified 122 genes with somatic mutations in breast cancer through large-scale DNA sequencing two of the 11 (18%) primary breast cancers had a heterozygous missense mutation in CHD-518.

In a study by Xiao et al, none of the 38 primary tumors had somatic mutations in CHD-5, while one of the 17 breast cancer cell lines examined had a frame shift mutation. Among the SNPs detected in this study, some occurred frequently in cell lines but were not detected in primary tumors, including 529G>C (8/34 alleles or 24%) and 4715T>C (76%); others occurred frequently in the primary tumors but were not detected in any of the cell lines, including 2479C>T (30%) and 3436G>A (36%)19. The results were consistent with another report, by Gorringe et al, in which no mutations were detected in 60 breast cancer samples while three mutations were identified in 123 ovarian cancer samples19, indicating that, although mutation of CHD- 5 could occur in breast cancer, its frequency is rather low in primary tumors. In this study, there was no association between SNP 4615T>C in CHD-5 gene and PA. SNP was present in PANC-1 but heterozygosity was seen in MIA PaCa-2 cell line. The cell lines can be used for further research regarding early diagnosis and screening of PA in future.

CONCLUSION

Hence, we conclude that SNP 4615T>C (Ser1539Pro) in tumor suppressor gene CHD-5 was present in PA. The similar results have been seen in cell lines also. But there was no significant association between SNP and PA.

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