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Tropical Chronic Pancreatitis (TCP): A Cytogenetic Study in Patients Residing in and around Coimbatore City, Tamil Nadu

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ABSTACT Tropical chronic pancreatitis (TCP) is a juvenile form of chronic calcific non- alcoholic pancreatitis found almost exclusively in tropical countries. This study was performed to find out the chromosomal aberrations in chronic pancreatitic patients of Coimbatore city, south India. 41 tropical chronic pancreatitis patients were examined for chromosome abnormalities using standard procedure of analysis from peripheral blood leukocyte culture using trypsin G – banding. The patients and the control set of individuals displayed different types of chromosomal abnormalities such as deletions, translocations, inversions and mosaicism. Familial transmission of chromosomal aberration was detected in 6 TCP patients and abnormalities in chromosome 7 were found in seven patients including the familial cases. The etiology of the disease needs to be studied, taking geographical location of Coimbatore city also into consideration.

INTRODUCTION

Pancreatitis is an ubiquitous disease with countless clinical etiologies (Sleisenger and Fordtran 1978) and occurs in all parts of the world. It affects both sex of all age groups, races and ethnic groups. We do not have data on distinguishing features by gross or microscopic examination to differentiate different types and on the etiology (Kaplan and Wheeler 1983; Kaplan 1986).

Tropical chronic pancreatitis (TCP) is a juvenile form of chronic calcific non alcoholic pancreatitis found almost exclusively in tropical countries. TCP is characterized by recurrent abdominal pain, exocrine pancreatic insufficiency, pancreatic calculi and diabetes, symptoms may vary in different patients (Barman et al. 2003). In India, tropical pancreatitis is widely prevalent in southern part of the country and in Orissa. The prevalence of tropical pancreatitis is about 126/ 100,000 population in southern India. It occurs usually in young people, involves the main pancreatic duct and results in large ductal calculi. Clinically, more than 90% of patients present themselves with abdominal pain. About 25% of patients develop diabetes which generally requires insulin for its control but is ketosis resistant. Painless diabetes is another clinical presentation in some patients. Most patients develop malnutrition during the course of the disease. Steatorrhea is less common (Tandon and Garg 2004). The etiology of TCP is possibly multifactorial. During course of chronic pancreatitis the secretory capacity of pancreas and luminal digestion decreases (Anderson et al. 1994).

In almost all countries where tropical pancreatitis has been reported, cassava is one of the staple items of diet. However, evidence to support the nutritional origin and the cassava hypothesis for TP remains inadequate (Balakrishnan et al. 2006). Pancreatic calcification associated with malnutrition and diabetes mellitus was first reported by Zuidema (1959). The most common cause of pancreatic exocrine deficiency in chronic pancreatitis is a result of two major problems namely, poor nutrition and social embarrassment. Tropical pancreatitis is seen among the deprived sections of people in developing countries. They eat protein and calorific deficient diets and show signs of under nutrition. A characteristic feature of chronic pancreatitis is progressively decreasing pancreatic enzymes output, with consequence of increasing nutrient malabsorption (Di Magno et al. 1993). Bell et al. (1984) found that fibro calculus pancreatic diabetes arises in an individual who has part of the pre –existing genetic susceptibility to either type 1 or type 2 diabetes and evidence of tropic chronic pancreatitis.

A genetic etiology of this disease has been suggested by Pitchumoni (1970), Braulke et al.

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(1994) and Raja and Sasikala (1995) who observed an increasing clustering of fibrocalculus pancreatic diabetes within families. Geographical and even familial clustering of patients with tropical pancreatitis has been reported, particularly from the state of Kerala (Pitchumoni 1970). One of the very interesting observations in cases of tropical pancreatitis is familial clustering. Raja and Sasikala (1995) showed the disease in three cases, twins of many siblings and in a father and his son. The high incidence of tropical pancreatitis in Keralites in and outside Kerala and its rarity among non – Keralites living in the state points indicates genetic predisposition.

The etiology of tropical chronic pancreatitis still remains to be understood. Detailed dietic and enzymatic factors need to be explored to understand the etiological genesis of tropical chronic pancreatitis especially in the population of Coimbatore city, as it borders Kerala and may provide insights in to the disease.

MATERIALS AND METHODS

Subjects for the present study were selected using questionnaire from the general public residing in and around Coimbatore city, Tamil Nadu, India. The experimental subjects of the experimental group were categorized on the basis of duration of disease such as category 1(<2 years), category 2 (3-4 years) category 3(5-6 years) category 4(7-8years) and category 5(9-10 years). Again the subjects in each category were divided into four groups depending on their age as Group I (<15 years), Group II (16-30 years) Group III (31-45 years) and Group IV (46- 60< years). Equal number of mentally normal and physically healthy subjects of matching age group constituted the control set.

Blood was collected from the individuals of the experimental and the control group in heparinized tubes and subjected for chromosomal analysis. Leukocyte cultures were set up follow-ing standard procedures (Hoyos et al. 1996). 0.5 ml blood was added to 4.5ml RPMI 1640 medium supplemented with 10% fetal bovine serum, 2mM 1 – glutamine, 1% streptomycin-penicillin, 0.2ml phytohemagglutinin, and was incubated at 37°C. After 71 h, cultures were treated with 0.1 g/ml colcemid to block cells in mitosis. Lymphocytes were harvested after 72 hrs by centrifuging cells to remove culture medium (800-1000 rpm), followed by addition of hypotonic solution (KCl 0.075 M) at 37 °C for 20 min to swell the cells, and treating twice with Carnoy's fixative (3:1 ration of methanol : acetic acid). Slides were carefully dried on a hot plate (56 °C, 2 min). Then the slides were stained using the trypsin G – banding technique and analyzed at 100X to scan and to record the incidence of aberrations.

RESULTS

The observations are presented in table 1. Twelve of 41 patients and 4 individuals of control group displayed different chromosomal aberrations such as deletions, translocations, inversions and mosaics. Familial transmission was noted in 6 patients with two instances involving fathers and sons. In the case of fathers displaying a trans-location of short arm of chromosome 5 and a long arm of chromosome 7[46, XY, t (5p; 7q)] was observed; whereas the sons exhibited a deletion of long arm of chromosome 7(46, XY, del 7q-). Similarly a grandmother of age 53 years had exhibited mosaicism [46, XX/46, XX, del (6q-)] for the deletion of long arm of chromosome 6 (Fig. 1). More than 85% of cells showed normal karyotype

Table 1: Types of chromosomal aberrations found in TCP patients of Coimbatore city

S. No.	*Type of chromosomal aberrations in patients with TCP*	*Duration of TCP and group and category to which the patient belongs*	*Relationship*
1.	46,XY, tc(Dq:Gq)	Category 3 Group III	
2.	46,XX/46,XXinv(9)	Category 3 Group III	
3.	46,XX,inv(9)	Category 4 Group III	
4.	46,XY,del(7p-)(3 cases)	Category 5 Group II	
5.	46,XY,t(5p:7q)	Category 5 Group IV	Two Fathers and their
6.	46,XY,del(7q-)	Category 2 Group II	respective sons
7.	46,XX/46,XX,del(6q-)	Category 4, group IV	A grandmother and
8.	46,XX,del(6q-)	Category 2 Group I	her grand daughter

Category 1 (<2 years), category 2(3-4 years) category 3(5-6 years) category 4 (7-8 years) and category 5 (9-10 years).
Group 1 (<15 years), Group II (16-30 years) Group III (31 – 45 years) and Group IV (46 – 60< years).

(46XX) and remaining 15% were found with the deletion of long arm of chromosome 6. Her granddaughter displayed a deletion of part of long arm of chromosome 6(46, XX, del 6q-).

Fig. 1. A female patient showing deletion in log arm of chromosome 6

A deletion of short arm of chromosome 7(46, XX, del (7q-)) was observed in three females of category 5 and Group II and a female control in Group III. An inversion of chromosome 9(46, XX, inv 9) was detected in a female with duration of disease between 7 and 8 years and in a male control of category 4 and Group IV (Fig. 2). Another control in Group IV showed a deletion in short arm of chromosome 5(p-). A mosaic type was found in a female of category 3, Group III. The normal chromosome complement was found in 70% of the cells and the remaining 30% were with the defective chromosomal complement namely the inversion of chromosome 9(46, XX/ 46, XX, inv 9). Translocation between the long arm of D and G group chromosomes was detected in a male aged 40 years (46, XY, tD; G). It can be inferred that a familial transmission possibly exists among TCP patients and this needs to be confirmed employing a large number of samples.

Fig. 2. A female patient displaying inversion in chromosome 9

DISCUSSION

Tropical pancreatitis is seen in certain areas of Indonesia, India, and Africa. The disease typically presents in childhood, with diabetes, abdominal pain, steatorrhea, malnutrition, and diffuse pancreatic calcifications. Malnutrition appears to be an important cofactor in this disease, as may be the presence of toxic metabolites of the dietary staple cassava. Chronic pancreatitis seen in tropics produces limited B-cell destruction in an individual with genetic predisposition to diabetes mellitus. A genetic etiology of this disease has been suggested by Pitchumoni (1970); who observed an increased clustering of FCPD within families. Balakrishnan (1987) reported the disease in three twins, many siblings and a father and son. It has also been noticed that calcific pancreatitis and non calcific pancreatitis, pancreatic carcinoma and diabetes mellitus run in the same families. In a study carried out by these on 155 patients, 39(29%) gave history of either diabetes mellitus or pancreatitis in other members of the family. In another study by them with family members of 24 patients with calcific pancreatitis, there was tropical pancreatitis in 12, diabetes mellitus in 16, and carcinoma in 1. In a family of 15 patients of non calcific pancreatitis there was a twin sister with calcification, 2 members had tropical pancreatitis, 1 with carcinoma and 11 with diabetes mellitus. .

Numerical chromosomal abnormalities have been reported from alcoholic pancreatitis patients by De Torok (1972), Raja and Sasikala (1995) and by Raja et al. (1995). Norappa *et al.* (1980) noted that the most common type of aberration among the Acute Alcoholic Pancreatitis (AAP) patients were chromosomal gaps and breaks, but Badr and Hussain (1982) recorded dicentrics as the most prevalent type of chromosomal abnormality in their study of AP patients. Slacik et al. (1976) observed chromosome type and chromatid type of aberrations induced by monofunctional and bifunctional alkylating agents *in vitro* leukocyte cultures. Only few studies have been carried out on the cytogenetic aspects in tropical pancreatitis patients.

In the present study, all the 41 patients with TCP were subjected to chromosomal analysis along with the respective controls.

Two fathers grouped under 4 of category 5 displayed and translocation of short arm of chromosome 5 and a long arm of chromosome 7(46, XY, t5p; 7q); whereas their sons grouped under II exhibited a deletion of long arm of chromosome 7(46, XY, del(7q)). A deletion of short arm of chromosome 7 was observed in three females in the age group of 16-30 years with 9-10 years of disease period.

Whitcomb et al. (1996) compared micro satellite and cytogenetic maps of long arm of chromosome 7 and indicated that hereditary pancreatitis locus most likely falls within 7q 35. This report has been correlated with findings of abnormalities on chromosome 7 in TCP patients in the present study. PRSS 1, a gene localized to the T cell receptor beta locus on chromosome 7 has also been known to be affected in pancreatitis (Leger et al. 1962).

In our study familial transmission was noted in 3 cases, twice in a father and son and once in a grandmother and her granddaughter.

Another argument in favour of genetic factor is the finding of tropical chronic pancreatitis and calcifying pancreatitis in families. It is not a matter of large families with numerous cases but two cases in the same family, uncle and nephew among the patients, 2 brothers (Fitzergerald et al. 1963) father and daughter (Kotch and Bohn 1963) mother and daughter (Gee vergheese et al. 1962). Balakrishana (1984) found in 100 cases of calcific pancreatitis, three families in which 2 brothers, a father and son and a brother and sister were affected.

In our study chromosome 7 seems to be a major site for aberrations related to this form of pancreatitis. The disease also seems to manifest itself in families.

Subjects grouped under category one (duration of TCP < 2 years) did not show abnormal chromosomes. It is therefore assumed that the chromosome type aberrations found in cells of TCP patients in the present study may be due to such probable breaking effect of metabolites attributed to malnutrition and enzymatic deficiency.

Geographical and even familial clustering of patients with tropical pancreatitis has been reported particularly from the state of Kerala. There may be a possible ethnic predisposition to the disease as Coimbatore borders Kerala state. To confirm it further genetic studies are required and city of Coimbatore provides an excellent opportunity to unravel the complex issue.

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