JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

STUDY OF GENETIC VARIABILITY IN TRIBAL POPULATION OF KISHANGANJ DISTRICT (BIHAR, INDIA) BASED ON PHENYLTHIOCARBAMIDE (PTC) TASTE SENSITIVITY

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ABSTRACT

The genetic variability on the basis of genetic marker phenylthiocarbamide (PTC) was studied in three demes selected from Santhal, Munda and Oraon tribal populations of district Kishanganj of Bihar. The marker divides populations in taste sensitive controlled by dominant gene and taste blind controlled by recessive gene. The identification of population as taster and non-taster is based on the method of Harris and Kalmus. The study was based on total sample size 322 randomly selected from three administrative blocks Kishanganj, Bahadurganj and Pothia; in which 222 were Santhal, 50 were Munda and 50 were Oraon.

The gene and allelic frequencies were determined by Hardy Weinberg Law (HWL) and the gene diversity indexes were calculated on the method as suggested by Nei. The threshold level of PTC does not show any variation among demes and in between gender but the allelic frequency of dominant and recessive genes represent significant variations among three populations considered in present study. The dominant gene frequency in Santhal, Munda and Oraon is 51%, 28% and 37% respectively. In all these three demes the heterozygous dominant is more prevalent (50 % in Santhal, 40% in Munda and 47% in Oraon) and suggests that both taster and non-taster alleles have survivorship advantage in existing environmental conditions of the population.

Santhal represents allelic equilibrium of both dominant and recessive genes, since it is a Mendelian trait and represent 1.04: 2: 0.96 genotypic ratios with corresponding allelic frequencies as 26%, 50% and 24%. The findings suggest normal pattern of allelic distribution and balancing selection of genes. Munda and Oraon represent genotypic ratio as 0.32 : 1.6 : 2.28 and 0.52 : 1.88 : 1.6 with allelic frequencies as 8%, 40% and 52% and 13%, 47% and 40% respectively. The findings suggest that the distribution of homozygous dominant is skewed in both populations and non-taster allele is fixed. The probable causes are discussed in paper but it requires more extensive research for final conclusion.

The different parameters of gene diversity were calculated as H_T = 0.4738, H_S = 0.4557, D_{ST} = 0.0181 and G_{ST} = 0.0383.

KEY WORDS – Allelic frequency, variability, selection, PTC sensitivity, Tribal population, Kishanganj.

INTRODUCTION

The well recognized genetic marker phenylthiocarbamide (PTC) was reported serendipitously with bitter taste and categorized population into taste positive and taste negative groups [1]. Sometimes the sweet, salty or sour tests were also reported[2]. The trait follows monohybrid Mendelian pattern of inheritance [3, 4, 5] and controlled by two alleles, one dominant (T) and one recessive (t). The tt individuals are taste blind and T_ individuals are taste sensitive. The taste sensitivity is used by different researchers for the study of variability among populations throughout the world. It has been estimated that 70% population of the globe are PTC positive. In different continents the percentage of sensitivity is varied, taste negative populations are reported 40% in India, 30% in US, 6 – 23% in China and 3% in West Africa [6,7,8]. Within Indian population the percentage of taste negative represents variation in different endogamous populations differ on the basis of ethnicity, race and caste [9, 10, 11, 12].

The Mendelian pattern of inheritance and bitter taste of PTC attracted many researchers to work on allelic frequency of genes in different populations and describe its role in evolution particularly related with feeding habit. Although PTC is not reported from any known human food, its chemical moiety (- N = C = S) responsible for bitter taste common in many plants. The concept of balancing selection for the evolution of PTC tasting was at first proposed by geneticist R. A. Fisher and colleagues [13]. Later on many workers studied the evolutionary significance of bitter taste in same natured chemicals present in edible substances and published their findings related with increase of survivorship and gene adaptivety in primates and its global distributed with time [14, 15, 16, 17, 18].

Humans possess thirty three bitter taste genes referred as TAS2Rs; among which 25 are presumed to be functional genes and 8 are pseudogenes. The majority of bitter taste genes are found either on 12th chromosome and 7th chromosome with a single gene on chromosome number 5th. The TAS2Rs can be again varied on the basis of their association with taste receptors and group of chemicals. The PTC and related chemicals are associated with taste receptor coded by TAS2R38. This gene represents seven haplotypes at population level; in which two PAV (Proline – Alanine – Valine) and PVI (Alanine – Valine – Isolucine) are widely divergent [19, 20, 21, 22].

MATERIAL AND METHODS

STUDY AREA: The three administrative blocks Kishanganj, Bahadurganj and Pothia of district Kishanganj were selected for the sampling in present study. The district constitutes north eastern boundary of the Bihar and geographically covers 25^{0}21’ – 26^{0}30’ north latitude and 87^{0}7’ – 88^{0}19’ east longitude. Its bordering district in west is Araria and in south west is Purnea. In east, north east and north it is surrounded by Uttar Dinajpur, Darjeeling of West Bengal and Nepal respectively.

SUBJECT AND SAMPLING METHODS: The subjects selected for the study were tribal populations. They were sampled from all the three administrative blocks. An ethical clearance (letter number 1498 dated 14.07.2015) was obtained for the study on tribal populations in the district from Civil

Surgeon cum Chief Medical Officer, Sadar Hospital, Kishanganj. The total tribal population in the district is 64,224 as per 2011 census and represented 4% of the total population. The endogamous tribal populations selected for study were Santhal, Munda and Oraon. The total subjects included in study were 322 in which 222 were Santhal and 50 each were Munda and Oraon since Santhal represented ¾ tribal populations of the district whereas others two were less in number and confined to small pockets, more number of Santhal was included to neutralize the statistical biasness.

The stratified random sampling method was used for the selection of subject in the study. The inclusion of person in the study was avoided when he or she reported the common lineage to minimize the calculation biasness in genetic parameters. Santhal was present in all the blocks selected for the study whereas Munda and Oraon were mainly confinded in Pothia. The persons were included in field study after obtaining his/her consent or consent of guardian in the case of minor (less than 10 years). The personal details of each subject included in the study were recorded in pre- determined questionnaire

TASTE SENSITIVITY – The taste sensitivity test was based on the serial dilution method described by Harris and Kalmus [23] except omission of 14 numbered working solution and use of distilled water. A stock solution (0.13 % or 0.13 gm/100 ml of water) numbered – 1 of PTC was prepared and serially diluted to the 13 numbered of working solution. During field visit a randomly selected person was tested sequentially with lower concentrated to higher concentrated working solution (13 to 1). The lowest concentration tasted by a subject was considered as threshold value and the person was considered as taster. The person did not response to PTC solution was considered as non- taster. The tasters were further screened and finally conformed. The two bottles of distilled water and two bottles of same numbered working solutions were provided to them in very next day and the taste sensitivity was recorded further.

STATISTICAL ANALYSIS – The gene or allelic frequency was calculated on the basis of Hardy Weinberg Law (HWL), where T (dominant) represented to taster (p) and t (recessive) represented to non-taster (q). The gene diversity or heterozygosity and its components were analyzed on the basis of method described by Nei [24]. The average gene diversity of the total population (H_T) can be expressed as $H_T = H_S + D_{ST}$. Where, H_S and D_{ST} are the average gene diversities within and between subpopulations respectively. $D_{ST} = G_{ST} \times H_T$, where G_{ST} is gene differentiation relative to total population. The mathematical and statistical parameters were calculated in MS-Excel program bases on standard text book [25].

OBSERVATION AND DISCUSSION

In the present study the pooled allelic frequency of taster gene was observed as 0.4456 (~45%) for total tribal populations residing in district Kishanganj, Bihar. The genotypic frequencies for homozygous dominant, heterozygous dominant and recessive were observed as 0.1986 (~20%), 0.4941 (~49%) and 0.3073 (~31%) respectively. The gene frequencies of Santhal, Munda, and Oraon selected for the study were calculated as 0.51 (~51%), 0.2789 (~28%) and 0.3679 (~37%) respectively for taster gene. The observed allelic and genotypic frequencies for homozygous dominant, heterozygous dominant and recessive individuals in all these three demes are presented in table – 1. The present study represents that there is a variation in taster and non-taster allelic and genotypic frequencies among these three populatios selected for study in Kishanganj district. However the findings represent similarities with some earlier published works. The frequency for taster is

reported about 0.5 among European populations, 0.55 – 0.95 in Mongoloid population of East Asia and South East Asia and 0.59 – 0.67 among Tibetan population of North India [26, 27, 28]. Among Indian population the average frequency of taster gene is 45.7% (p=0.457); varies from 10.8% (p= 0.108) in Munda of Ranchi, Jharkhand to 91.2% (p=0.912) in scheduled caste in Andhra Pradesh [9, 10, 11, 12, 29]. The Oraon of Jharkhand has 24.7% frequency of T allele [30]. The findings represent that the allelic frequency of dominant (p) and recessive (q) genes are in equilibrium in Santhal whereas the recessive gene (q) is more prevalent in Munda (0.7211) and Oraon (0.6324). The chi square value (χ^2), at 0.05 level of significance, represents non - significant variation in genotypic frequencies in Santhal ($\chi^2 = 0.15015$, df = 1) whereas the Munda ($\chi^2 = 19.44$, df = 1) and Oraon ($\chi^2 = 6$, df = 1) represent significant variation. The significant difference in genotypic variation is observed either due to genetic drift or adaptation with respect to specific diet or sample fluctuation as the area under study is dominated by Santhal population and they represent 75% of total tribal populations of the district and the Munda and Oraons are very small in number migrated here from Chhotanagpur before 150 – 200 years. However further study on Munda and Oraon is suggested for the final conclusion.

The PTC threshold value (mean ± SD) was observed 6.18 ± 2.96 for total tribal population of Kishanganj. Independently the threshold value was observed 5.97± 3.03, 6.25 ±2.89 and 6.3 ±2.38 for Santhal, Munda and Oraon respectively. In general the threshold value is more in female than male population (table). The findings suggest that there is no difference of threshold value among all these three demes selected for the study. In term of gender, the variation is not significant as reported by some workers on the basis of sex hormone. The threshold of PTC represents bimodal nature among population [4, 5].

The observed values of phenotype, genotype and gene frequencies for all these three populations are presented in table – 2 and graph – 1. The hetyerozygotic frequency was observed 49.41% for total tribal population whereas Santhal, Munda and Oraon represented 49.98 %, 48.01 % and 46.49 % of heterozygous populations respectively. The different components of gene diversity were calculated. The value of average gene diversity in total population (H_T) was calculated as 0.4738, whereas the value of average gene diversity within population (H_S) was calculated as 0.4557. The value of average gene diversity between populations (D_{ST}) was calculated as 0.0181. The value of coefficient of gene differentiation (G_{ST}) was observed as 0.0383. The values represent that the inter-population gene diversity at PTC locus is small.

The findings suggest that the heterozygous individuals are more prevalent than homozygous individuals followed by homozygous recessive individuals except in Santhal. The more prevalence of heterozygous suggest that both taster and non-taster alleles of PTC have survivorship advantage. Thus they might govern a fitness advantage by regulating the intake of more diversified bitter compounds or food items than homozygous individuals [22]. The allelic frequency of Santhal represents balancing selection and the PTC taste locus represents normal pattern of distribution (1:2:1) in Kishanganj. It indicates that the population enjoyed a selective advantage over both the homozygotes. The similar type of observations has reported earlier by other workers in different populations and Chimpanzee [13, 21, 26]. The earlier works have also suggested that the natural selection can also be an important driving force in evolution but it is slow [27].

The two other tribal demes of Kishanganj do not represent symmetrical or normal pattern of genotypic frequency but they represent skewed nature for homozygous dominant. In both demes the recessive individual are more prevalent as Munda and Oraon represent genotypic ratio as 0.32 :

1.6 : 2.28 and 0.52 : 1.88 : 1.6 with allelic frequencies as 8%, 40% and 52% and 13%, 47% and 40% respectively. In addition with the balancing selection explained above, other hypotheses responsible for fixation of gene in any population are positive selection [28] or demographic events like a series of genetic drifts and population migration with a relaxation of selective forces. The PTC is not reported from natural food or natural products but the PTC like taste response is observed for l-5-vinyl-2 thio-oxazolidone and reported from some natural edible plant products like cabbage and rapeseeds. If the plant products help in the increase of survivorship of population, the non-taster allele have reached fixation. Hence the diet pattern with respect to surrounding of population is one of the major factors for the fixation of taste allele. Some worker suggest that the PTC taste sensitive components of food may interfere in metabolic activities of body and affect to rate of survivorship and ultimately to determine the rate of fixation of allele in population [15]. It has been reported that less iodine in diet tasters will be selectively favored whereas in excess of iodine non tasters will be favored [29]. The taste sensitivity has also role in the prevention of disease. It has been suggested that the non-tasters have selective survivorship advantage in area where malaria is prevalent as they can ingest more cyanide containing plants which reduce the severity of *Plasmodium* infection [14, 15, 16]. Here we discuss on the different factors may have role in the fixation of more recessive allelic in Munda and Oraon and for the determination of pin point factor more extensive research work is required.

CONCLUSION

After the observation and discussion it has been concluded that the threshold level of PTC is same in all the three tribal populations of Kishanganj as well as in both male and female and it is not affected by ethnic variation and gender. But the three tribal populations represent variation in allelic frequencies due to their different parental lineage and endogamy. The allelic and genotypic frequency of Santhal evolutionarily shows that they are in allelic equilibrium and represent balancing selection. The allelic and genotypic frequencies of Munda and Oraon represent that non taster gene is selectively fixed in population. It might be caused due to genetic drift from parental stock, specific diet pattern increases reproductive fitness, selective operation of nature on particular locus etc. But for the proper analysis of causative factor of such fixation a more extensive research is necessary.

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TABLE: 1 : REPRESENTS DISTRIBUTION OF TASTER AND NONTASTER GENES IN TRIBAL POPULATIONS OF DISTRICT KISHANGANJ, BIHAR (INDIA)

TRIBE	SAMPLE SIZE	OBSERVED VALUE		PERCENTILE		ALLELIC FREQUENCY		GENOTYPIC FREQUENCY			χ^2 value = 3.841 at p 0.05 level	
		TASTER	NON TAST	TASTER	NON TAST	p(T)	q(t)	TT	Tt	tt	χ^2 values	df
SANTHAL	222	169	53	0.7613	0.2387	0.51	0.49	0.2601	0.4998	0.2401	0.15015	1
MUNDA	50	24	26	0.48	0.52	0.2789	0.7211	0.0779	0.4022	0.5199	19.44	1
ORAON	50	30	20	0.6	0.4	0.3679	0.6324	0.1313	0.4652	0.3999	6	1
TOTAL	322	223	99	0.6925	0.3074	0.4456	0.5544	0.1986	0.4941	0.3073	5.67	1

TRIBE	THRESHOLD VALUES (MEAN ±SD)		
	MALE	FEMALE	TOTAL
SANTHAL	5.34±2.98	6.68±2.94	5.97±3.03
MUNDA	5.91±2.12	6.54±3.48	6.25±2.89
ORAON	5.73±2.53	6.63±2.64	6.3±2.38
POOLED	5.53±2.82	6.66±2.89	6.18±2.96

TABLE :2: REPRESENTS PERCENTAGE ALLELIC AND GENOTYPIC FREQUENCIES IN ALL THE THREE TRIBAL POPULATIONS SELECTED FOR THE STUDY IN KISHENGANG DISTRICT (BIHAR)

TRIBE	FREQUENCIES (%)	TT	Tt	Tt	tt
SANTHAL	Genotype	26.01	24.99	24.99	24.01
	Phenotype	75.99			24.01
	Heterozygotic		49.98		
	p/q Ratio	51		49	
MUNDA	Genotype	7.78	20.1	20.1	51.99
	Phenotype	48.01			51.99
	Heterozygotic		40.23		
	p/q Ratio	28		72	
ORAON	Genotype	13.52	23.24	23.24	39.99
	Phenotype	60.01			39.99
	Heterozygotic		46.49		
	p/q Ratio	37		63	
POOLED	Genotype	19.86	24.71	24.71	30.72
	Phenotype	69.28			30.72
	Heterozygotic		49.41		
	p/q Ratio	45		55	

GRAPH:1: REPRESENTS THE ALLELIC AND GENOTYPIC FREQUENCY DISTRIBUTION IN ALL THE THREE DEMES STUDIED FROM KISHANGANG DISTRICTOF BIHAR, INDIA