

<b>The International Journal of Frontier Sciences</b>	
<b>Dichromacy: Colour Vision Impairment and Consanguinity in Heterogenous Population of Pakistan</b>	
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<b>Abstract:</b>	
<p><b>Background and Objectives:</b> Dichromacy, an X-linked recessive disorder is identified worldwide, more in males than females. In European Caucasians, its incidence is 8% in males and 0.5% in females. In India, it is 8.73% in males and 1.69% in females, and in Iran, it is 8.18% in males and 0.43% in females. Population based epidemiological data about dichromacy in different ethnic groups in Pakistan is not available. The aim of this study was to find out the population prevalence of inherited red-green dichromacy in a heterogenous population of the district of Chiniot, Punjab, Pakistan, and to determine the impact of consanguinity and ethnicity.</p> <p><b>Methods:</b> In this cross-sectional study, boys and girls of the higher secondary schools were examined in the three tehsils of district Chiniot. Pseudoisochromatic Ishihara Test has been employed for detection of dichromacy in the study population. The sample size was calculated statistically as 260, which was expanded to 705 and divided by population density of the three tehsils.</p> <p><b>Results:</b> Screening of 359 males and 346 females revealed 19 (5.29%) dichromat males and only 2 (0.58%) females. The study population belonged to 23 castes / isonym groups. The consanguinity found in the district of Chiniot is 84.82% and in the dichromat families, it is 85.71%, of which 52.37% are first cousin.</p> <p><b>Interpretation &amp; Conclusion:</b> The study has shown that the incidence of dichromacy could be reduced through genetic counselling.</p> <p><b>Keywords:</b> Chiniot, Consanguinity, Deuteranopia, Dichromacy, Ethnicity, Heterogenous Population, Pakistan, Protanopia, Punjab</p>	
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### Introduction:

Dichromacy is an X-linked genetic trait presenting red-green colour-blindness clinically. Human eye has two types of photoreceptor cells: rods and cones located in retina. Rod cells are responsible for discrimination of black and white light, while cone cells have three types of photoreceptors i.e., short, medium and long wavelength photoreceptors. Cone cells are responsible

for color discrimination. (1) Human eye detects and responds to light through a series of events called phototransduction. (2) Dichromacy may be deuteranopia, protanopia or tritanopia. Deuteranopia is the lack of function of middle wavelength photoreceptors, thus, do not discriminate red-yellow-green range of colour. Less severe form of deuteranopia is termed as deuteranomaly. Protanopia is the deficiency

in discrimination of red colour due to lack of function of long wavelength photoreceptor cells. Protanomaly is less severe form of protanopia like deuteranomaly. (1) A trichromat person (with normal colour vision) has three types of photoreceptors: blue, green and red. Each photoreceptor has capability to phototransduce about 100 gradations of colour and exponentially a trichromat can see up to 2.3 million colours. But a dichromat can only see about 10,000 colours (3, 4).

**Genetics:** Dichromacy, being an X-linked recessive disorder, affects males more than females because females have two gene alleles in contrast to males having only one. X and Y are sex chromosomes that make male and female karyotypes as XY and XX. In sex linked inheritance, both alleles are expressed in contrast to autosomal inheritance in which only dominant allele is expressed. Dichromat fathers inherit his trait to their daughters who also have trichromat allele (inherited by mother) and expressed in 50% of cone cells, a so-called carrier condition. Carrier females (of dichromacy) after marrying a trichromat man may inherit their dichromat X chromosome to her son (XY). Her son will be dichromat, but her daughters will again be carriers of the trait. A female can only be dichromat if both of her parents inherit their dichromat X chromosomes. This phenomenon is more prevalent in populations with higher consanguinity rates. (5) OPN1MW and OPN1LW are the genes responsible for deuteranopia (also deuteranomaly) and protanopia (also protanomaly) respectively. Both genes are located on the long arm of X chromosome in band 8 of region 2. Sixteen mutations are reported for the OPN1MW gene in Human Gene Mutations Database (HGMD) that are of different types including

missense, nonsense, insertions and deletions. Of these sixteen, only seven mutations cause deuteranopia. According to HGMD, fifty-five mutations of OPN1LW gene are reported and only 11 mutations are causing protanopia or protanomaly. Mutations in OPN1MW and OPN1LW other than mutations causing deuteranopia and protanopia are cause of altered absorption spectrum, blue cone monochromatism, Bornholm eye disease, cone-rod dystrophy, and X-linked cone degeneration.

**Worldwide Prevalence:** Worldwide dichromacy is present in approximately 7% of males and 0.5% of females, however, prevalence rates vary in different populations: China - 6.9% and 1.7% (6), France – 8.95% and 0.50% (7), Germany – 7.75% and 0.36% (7), Greece – 7.95% and 0.42% (7), India – 8.73% and 1.69% (8), Iran – 8.18% and 0.43% (9), Iraq – 8.47% and 1.37% (10), Israel – 10% in Ashkenazi Jews and 4% in Yemenite Jews (11), Korea – 5.9% and 0.4% (7), Nepal - 3.8% among males (12), Netherlands – 7.95% and 0.45% (7) Norway – 8.01% and 0.44% (13), Saudi Arabia – 5.85% and 0.75% (14), Switzerland – 7.95% and 0.43% (7), and Taiwan – 5.3% and 0.2% (7) among males and females respectively.

This study aims to find prevalence of dichromacy in a multi-ethnic heterogenous population in Pakistan. History of consanguinity is also recorded and reported in association with dichromacy.

### **Materials and Methods:**

**Settings:** Study was conducted at Human Genetics and Molecular Biology Department of University of Health Sciences, Lahore, Pakistan. Individuals screened for dichromacy were high school students from District Chiniot of Pakistan.

**Study Design:** This was a cross-sectional study.

**Sampling Technique:** Cluster sampling was used to collect primary data.

**Sample Size:** Total of 705 individuals participated in the study: 359 were males and 346 were females.

**Study Instrument:** A pre-tested questionnaire (Supplementary File 1) was used to collect data for current study.

**Dichromacy Screening Technique:** Pseudoisochromatic Ishihara Test (38 Plates Edition) was used as a screening tool. Ishihara Test was used for screening school volunteers in well day-lighted class rooms having windows and ventilators giving access to natural day light to class rooms. No other screening tool was used in this field study.

**Target Population:** Six high schools in District Chiniot (Punjab, Pakistan) were selected after principal permission to study genetic trait of dichromacy (Supplementary File 2). Students in the secondary classes could only participate in the study. The data for their origin, ethnicity and consanguinity

among parents were recorded in questionnaire.

**Inclusion Criteria:** Both male and female students from secondary classes lying in age group of 14-18 years without any vision impairment were included.

**Exclusion Criteria:** Individuals with congenital vision defects other than dichromacy were excluded.

**Statistical Analysis:** The data, recorded in SPSS (Version 20.0) for analysis purpose, were analysed for descriptive statistics. Sampling distribution frequencies, gender distribution among studied population and frequency of dichromacy among different castes were calculated. The percentage of dichromacy among males and females is calculated separately. In addition, percentage of dichromacy in each tehsil and its distribution by gender were also calculated. Chi Square Test was used to measure the association between dichromacy and consanguinity. This (chi square test) resulted in reporting the associations in terms of Odds Ratios and their 95% confidence intervals giving the Chi-square with its p-value.

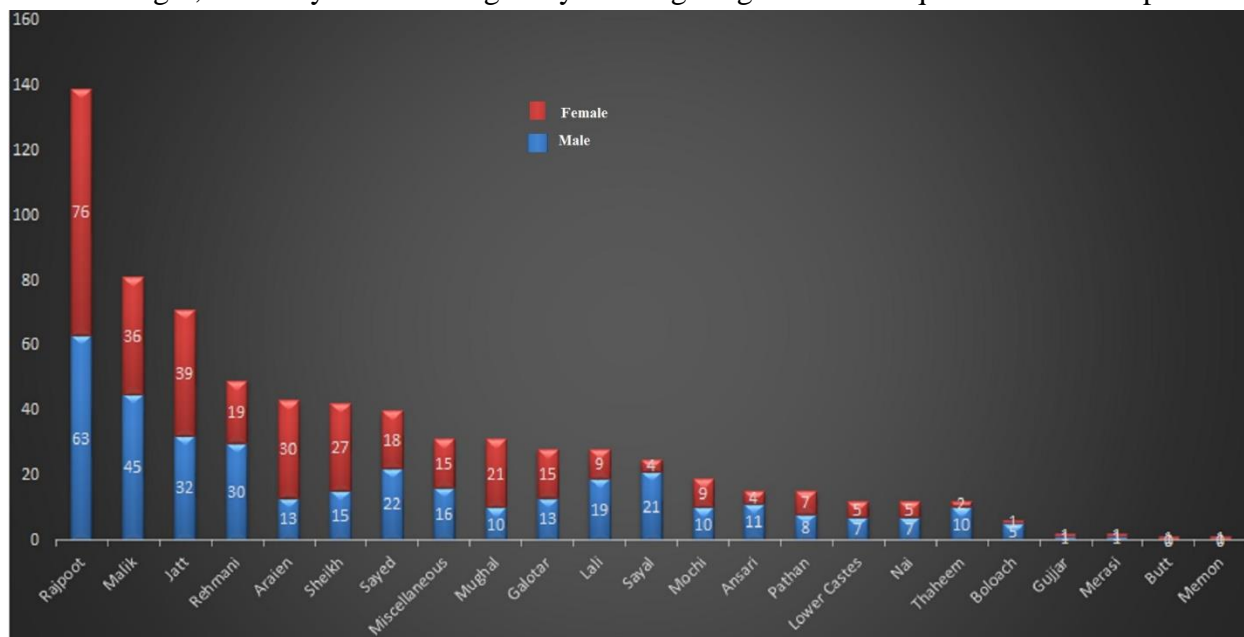


Figure 13: Distribution of different castes in study population

Frequency plot of participating castes is shown using the grouped bar chart.

**Ethical Consideration:** The study was approved by Ethical Review Committee of University of Health Sciences, Lahore-54600, Pakistan. Participating volunteers took an informed consent form.

**Results:**

A total of 705 District Chiniot residents (volunteers) were examined for dichromacy. Of these, 239 were from Bhowana, 261 from Chiniot, and 205 from Lalian. Among these 705 individuals, 359 (50.9%) were males and 346 (49.1%) were females. Of 359 males, 113, 131 and 115 were from Bhowana, Chiniot and Lalian respectively; and of 346 females, 126, 130 and 96 were from Bhowana, Chiniot and Lalian tehsils respectively. The data was analysed based on ethnic / isonym groups. Each caste was considered as a single group of isonyms with division of sub-castes. Thus, 21 major castes had been identified and included in the study from three different tehsils of

District Chiniot while two categories were defined as miscellaneous and minor castes. Miscellaneous castes are those castes not present frequently in the district of Chiniot. These are Alishrana, Chookhiya, Jopu, Kalas, Kariyala, Kula, Machi, Masoor, Matmal, Menkal and Wassi. Minor castes are listed as castes with low social background and included Changar and Muslim Sheikh (They are the aborigines of the land conquered by the Arians and branded as untouchable in Indian society). The castes, included in study, are presented in Figure 1, while distribution of dichromacy among different castes is demonstrated in Table 1.

A total of 21 cases of dichromacy were found in three tehsils: 9 from Bhowana (7 males and 2 females), 6 from Chiniot (males) and 6

from Lalian (males). The total percentage of dichromacy of the study population of the District Chiniot is found 2.98%. The prevalence (percent) of dichromacy among males is 5.29% and that of females is 0.58%. Tehsil wise prevalence of dichromacy in Bhowana, Chiniot and Lalian is 3.76%, 2.29% and 2.92% respectively. The percentage among males is 6.19%, 4.58% and 5.21% in Bhowana, Chiniot and Lalian respectively.

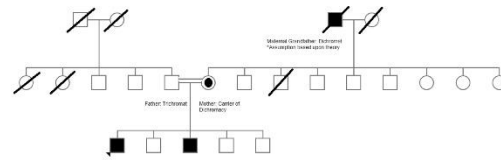


Figure 14: Pedigree of one male proband identified during study

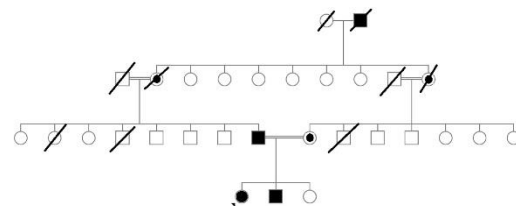


Figure 15: Pedigree of one female proband identified during study

Percentage among females of Bhowana, Chiniot and Lalian is 1.58%, 0.00% and 0.00% respectively. Of these dichromats, protanomaly was found in 1 individual, protanopia was found in 6 individuals, deuteranomaly was found in 2 individuals and deuteranopia was found in 6 individuals. There were six dichromats that couldn't be classified into protanopia/protanomaly and/or deuteranopia/deuteranomaly using Pseudoisochromatic Ishihara Test. The prevalence of dichromacy types among males and females is described in Table 2.

The consanguineous history of the parents of study subjects revealed 84.82%, which was

slightly higher in the parents of dichromats (85.71%), of whom 52.37% were first cousins. Interestingly, patrilineal dichromats

Consanguinity in parents of dichromats is shown in Table 3. Pearson Chi Square Test reported association between

Caste of Individuals	Gender of Individuals		Dichromat		Red-green Color Deficiency Percentage
	Male	Female	Male	Female	
<b>Ansari</b>	<b>11</b>	<b>4</b>	<b>1</b>	<b>0</b>	<b>6.67</b>
<b>Araien</b>	<b>13</b>	<b>30</b>	<b>0</b>	<b>0</b>	<b>0.00</b>
<b>Baloach</b>	<b>5</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0.00</b>
<b>Butt</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0.00</b>
<b>Galotar</b>	<b>13</b>	<b>15</b>	<b>0</b>	<b>0</b>	<b>0.00</b>
<b>Gujjar</b>	<b>1</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0.00</b>
<b>Jatt</b>	<b>33</b>	<b>39</b>	<b>1</b>	<b>0</b>	<b>1.38</b>
<b>Lali</b>	<b>19</b>	<b>9</b>	<b>2</b>	<b>0</b>	<b>7.14</b>
<b>Minor Castes</b>	<b>7</b>	<b>5</b>	<b>0</b>	<b>1</b>	<b>8.33</b>
<b>Malik</b>	<b>45</b>	<b>36</b>	<b>1</b>	<b>0</b>	<b>1.23</b>
<b>Memon</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0.00</b>
<b>Merasi</b>	<b>1</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0.00</b>
<b>Miscellaneous</b>	<b>10</b>	<b>15</b>	<b>1</b>	<b>0</b>	<b>4.00</b>
<b>Mochi</b>	<b>10</b>	<b>9</b>	<b>1</b>	<b>0</b>	<b>5.26</b>
<b>Mughal</b>	<b>10</b>	<b>21</b>	<b>1</b>	<b>0</b>	<b>3.22</b>
<b>Nai</b>	<b>7</b>	<b>5</b>	<b>0</b>	<b>0</b>	<b>0.00</b>
<b>Pathan</b>	<b>8</b>	<b>7</b>	<b>0</b>	<b>0</b>	<b>0.00</b>
<b>Rajpoot</b>	<b>67</b>	<b>76</b>	<b>7</b>	<b>1</b>	<b>5.59</b>
<b>Rehmani</b>	<b>30</b>	<b>19</b>	<b>0</b>	<b>0</b>	<b>0.00</b>
<b>Sayal</b>	<b>21</b>	<b>4</b>	<b>2</b>	<b>0</b>	<b>8.00</b>
<b>Sayed</b>	<b>23</b>	<b>18</b>	<b>1</b>	<b>0</b>	<b>2.44</b>
<b>Sheikh</b>	<b>15</b>	<b>27</b>	<b>1</b>	<b>0</b>	<b>2.38</b>
<b>Thaheem</b>	<b>10</b>	<b>2</b>	<b>1</b>	<b>0</b>	<b>8.33</b>
<b>Total</b>	<b>359</b>	<b>346</b>	<b>19</b>	<b>2</b>	<b>2.98</b>

were 33.33% and matrilineal were 19.04%. Second cousin marriages were 19.04%, and marriages in Bradri (same clan) were 19.04%. Inter-caste marriages were only 9.52%.

dichromacy and consanguinity (p-value is 0.003).

Pedigrees of the dichromat individuals were drawn to understand the pattern of inheritance. However, extended family exploration and identification of dichromats

**Table 2: Classification of Dichromacy in Study Population**

Dichromacy Type	Caste	Gender		Prevalence	
		Male	Female	Male	Female
Protanomaly	Ansari	1	0	0.28%	0.00%
Protanopia	Mughal Sayal Rajpoot	5	1	1.39%	0.28%
Deuteranomaly	Rajpoot	1	1	0.28%	0.28%
Deuteranopia	Lali Sayal Sayed Sheikh Rajpoot	6	0	1.73%	0.00%
*6 individuals couldn't be classified using Pseudoisochromatic Ishihara Test.					

were not possible because of far-off residences of relatives and other social issues in local context of the Pakistani population. Two pedigrees are part of this article as figure 2 and figure 3. Figure 2 is male proband and figure 3 is female proband.

**Table 3: Dichromacy in Relation to Consanguinity in Study Population**

Consanguinity among parents of study individuals	Dichromats
Maternal Aunt Cousin (Khalazad)	3
Maternal Uncle Cousin (Mamonnzad)	1
Paternal Aunt Cousin (Phuphizad)	0
Paternal Uncle Cousin (Chachazad)	7
Second Cousin	4
Distant Blood Relation	0
Same Sub-caste (Bradri)	3
Same Caste	1
Different Caste	2
<b>Total</b>	<b>21</b>

**Discussion:**

In Pakistan, the known percentages of dichromacy among males are 3.59% (15), 5.69% (16), 2.75% (17), 2.24% (18), 10.0% (19), 7.95% (20), and 3.1% (21) however, these show a great variation. The known percentages for females are 4.48% (18), 1.64% (19), and 1.39% (20). The current study and previous studies on Pakistani population are presented in Table 4. The data comparable to our study (5.29%) are from Quetta, Baluchistan and Southern Punjab populations. (16) The first study reported prevalence of 4.89% among the Punjabi males living in Quetta while our study finds it as 5.29%. The Quetta study reported prevalence of 5.16% and 9.68% for Pathan and Baloach ethnic groups respectively. However, we could not find any dichromat among the populations of Pathan

and Baloach in our studied population. However, these ethnic groups were in minority in study population. According to a study of Mian et al., and Munawar et al. prevalence of dichromacy is 3.59% and 3.1% in southern Punjab, while the current study population is from Central Punjab and both populations are diverse in ethnicity and origin. (15, 21)

In Karachi, Sindh study, the figure of 2.75% represents the prevalence of dichromacy among male students in 4 institutions. (17) This population is representing multi-ethnic isonym groups, not only of Sindh but also of all of Pakistan. And the studied

Population	Dichromacy Prevalence (%)		Study
	Male	Female	
Aesthetic Dentists, Karachi	10.0	1.64	Yousuf et al. 2015
Chiniot, Central Punjab	5.29	0.58	Current Study
Faisalabad, Central Punjab	2.24	4.48	Mughal et al. 2013
Karachi, Sindh	2.75	-	Siddiqui et al. 2010
Peshawar, Khyber Pakhtunkhwa	7.95	1.39	Khalid et al. 2017
Quetta, Baluchistan	3.59	-	Mian et al. 1991
South Punjab	5.69	-	Mian et al. 1994
South Punjab	3.1	-	Munawar et al. 2018

population is representing different races and ethnic origins, for which specific caste information is not available. While our study in Chiniot represents local urban and rural inhabitants who share close ethnicity and nearby geographic / climatic factors except Baloach, Memon, and Pathan. Baloach, Memon and the Pathan populations are far in ethnicity but live in the same geographical area. But these ethnic groups are also inhabitants of the district for generations, as

reported in population censuses by national census bureau.

The third study, conducted in medical colleges of Faisalabad, Punjab, recorded 2.4% of male and 4.48% of female dichromats. (18) This study represents the sample of students from different ethnic and geographical backgrounds of the Punjab. On the other hand, the figure of 4.48% for dichromat females seems not only quite high but erroneous, because it gives the figure of 2.2% for male dichromats, which is too low. If the figure for females is taken as correct, then the figure for males should have been at least 10 times higher than females. Dichromacy is an X-linked trait and its incidence is always higher in the hemizygous male sex.

The fourth study, conducted in a specific group of professionals, i.e., aesthetic dentistry practitioners of Karachi, gives dichromacy in 10.0% of males and 1.60% of females.(19) Karachi is the business hub of Pakistan. Thus, population of Karachi is a conglomeration of different ethnic, cultural and geographic origins. Only 100 males and 183 females were included in this study among which 13 (4.59%) were dichromats. However, presence of male (10.00%) and female (1.64%) dichromats was higher than expected. The information about the ethnicity and consanguinity is not available of the specialized group, which may have some sampling bias. Another similar study (Khalid et. al) reported prevalence of dichromacy as 7.95% and 1.39% among male and female dentistry students in Peshawar (20). Peshawar is the capital of Khyber Pakhtunkhwa Province. Khyber Pakhtunkhwa population is of Pashtun origin whereas in our study, any Pashtun (Pathan is not found to be dichromat, probably due to a limited sample.

Country/Population	Dichromacy Prevalence (%)		Study
	Male	Female	
China	6.9	1.7	Kilborn & Beh 1934 (6)
France	8.95	0.50	Birch 2012 (7)
Germany	7.75	0.36	Birch 2012 (7)
Greece	7.92	0.42	Birch 2012 (7)
India	8.73	1.69	Ahsana et al. 2013 (8)
Iran	8.18	0.43	Modarres et al. 1996 (9)
Iraq	8.47	1.37	Karim & Saleem 2013 (10)
Israel (Ashkenazi Jews)	10	-	Adam et al. 1967 (11)
Israel (Yemenite Jews)	4	-	Adam et al. 1967 (11)
Korea	5.9	0.4	Birch 2012 (7)
Nepal	3.8	-	Niroula & Saha 2010 (12)
Norway	8.01	0.44	Waaler 1927 (13)
Pakistan	5.29	0.58	Current Study
Saudi Arabia	5.85	0.75	Oriowo & Alotaibi 2008 (14)
Switzerland	7.95	0.43	Birch 2012 (7)
Taiwan	5.3	0.2	Birch 2012 (7)
The Netherlands	7.95	0.45	Birch 2012 (7)

This study provides population-based prevalence of dichromacy and is compared with prevalence of dichromacy in different populations of the world in Table 5. Our estimated percentage of dichromacy among males is 5.29%, while percentage of dichromacy in India is 8.73% (8) and in Iran 8.18% (9). The percentage of dichromacy in our study among females is 0.58% comparable to incidence of dichromacy among females in Iran (0.43%) (9). Indian Muslims reported dichromacy among females is 1.69% which is too high probably due to higher consanguinity rates. (8)

Among castes, the highest prevalence of dichromacy is found in Thaheem followed by Sayal, Lali, Ansari, Rajpoot, Mochi, Mughal, Sayed, Sheikh, Jatt and Malik. In other castes, which are present in the district of Chiniot, no individuals with dichromacy were found. This may be due to a smaller

number of samples representing that caste. It is planned to study all these castes with equally distributed sample size.

Consanguinity is an important part of rural culture in the Pakistani community thus significant negative impact on public health. (22, 23) A very first study conducted in 8 villages of 4 districts of the Punjab revealed 80% consanguinity with 100% intra-caste marriages. (24) In the current study population, 9.52% of marriages were inter-caste, although not ideal, but a sign of awareness.

Genetic counselling of dichromats is imparted by guiding them about their future marriages. Emphasis is placed on avoiding marriage to blood relatives and in particular to maternal cousins. In case of an unavoidable consanguinity, it is suggested that a pre-marital evaluation of the life



partner be performed for dichromacy and other genetic diseases.

### Conclusion:

Current study is significant for reporting the prevalence of dichromacy in local population consisting multiple isonyms. This study also showed association of dichromacy with consanguinity in studies population.

In this study, dichromacy is identified first time in the Central Punjab district, Chiniot and reported prevalence of dichromacy as 5.29% in males and 0.58% in females. The study showed the association of dichromacy with consanguinity.

**Suggestion:** It is suggested that children be tested for dichromacy at the time of admission to school and should be provided with customized teaching to manage learning barrier of dichromacy. It is known that dichromacy has a strong impact on the professional career of individuals. The dichromats should be counseled for their careers and adaptations to live a happy and peaceful life.

**Future Prospect:** Because of this pioneer study, we plan to investigate the prevalence of dichromacy with larger sample and in other ethnic groups in other provinces of Pakistan which may include molecular based genetic study of dichromacy.

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Govt. Girls High School, Lalian

Govt. Higher Secondary School, Bhowana  
Govt. Girls High School, Bhowana  
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**Conflict of Interest:** Authors do not have any conflict of interest.

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Supplementary File 1:

## Human Genetics & Molecular Biology Department University of Health Sciences Lahore

### Genetic Epidemiology, Risk Factors and Identification of Colour-blindness in Different Isonym Groups of District Chiniot, Punjab, Pakistan

Date: \_\_\_\_\_

No: \_\_\_\_\_

Name \_\_\_\_\_

Gender:

Male	Female
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Address:

\_\_\_\_\_

Age, (years): \_\_\_\_\_ Union Council: \_\_\_\_\_ Tehsil: \_\_\_\_\_

Mobile: \_\_\_\_\_ Email: \_\_\_\_\_

Caste: \_\_\_\_\_ Subcaste: \_\_\_\_\_

#### Consanguinity of parents: (Father married to)

<b>First Cousin</b>	Khalazad	Mamonzad	Phuphizad	Chachazad	
<b>Second Cousin</b>	Distant Blood Relation	Bradri	Same Caste	Different	Specify

Profession: \_\_\_\_\_ Family Occupation: \_\_\_\_\_

Marital status:

Married	Single
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Smoking status:

Current smoker	Former smoker	No history	Passive smoker	Hukka
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Tea consumption (green/black), cups/day: \_\_\_\_\_

Any other disease: if yes then specify:

\_\_\_\_\_

\_\_\_\_\_

# Ishihara Test:

<b>Plate 1</b>	<b>Plate 2</b>	<b>Plate 3</b>
<b>Plate 4</b>	<b>Plate 5</b>	<b>Plate 6</b>
<b>Plate 7</b>	<b>Plate 8</b>	<b>Plate 9</b>
<b>Plate 10</b>	<b>Plate 11</b>	<b>Plate 12</b>
<b>Plate 13</b>	<b>Plate 14</b>	<b>Plate 15</b>

**Remarks:**

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<b>Plate 16</b>	<b>Plate 17</b>

**Remarks:**

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**Association with different factors:**

(Fill only in case of colour-blind individuals)

Disease/Risk Factor	Yes	No	Don't know
Hypertension in family			
Diabetes in family			
Myocardial Infarction in Family			
Have you got TB anytime in your life?			
Any trauma to eye or nervous system			
Parkinson's Disease			
Kallmark's Syndrome			
Cataract in family			
Any other vision impairment			
Night Blindness			
Liver Cirrhosis			

**Pedigree analysis: (PTO)**


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For further questions contact Muhammad Shoaib Akhtar, Department of Human Genetics and Molecular Biology, University of Health Sciences, Lahore. Mobile 0313-7009201, Landline 042-9231304 Ext 320.

**Supplementary File 2:****Chiniot – Punjab - Pakistan**

Pakistan is sixth most populous country in the world with population exceeding 20.7 million. It has four provinces, i.e., Baluchistan, Khyber Pakhtunkhwa (KPK), Punjab, and Sindh. FATA<sup>1</sup> is living area of Pakhtoon tribes. Azad Jammu and Kashmir is a state of Pakistan, and Gilgit-Baltistan has provincial status. Population of Pakistan is ethnically very diverse. Punjab and Sindh Provinces have caste system with different ethnicity, while Baluchistan and Khyber Pakhtunkhwa have tribal system and both are different in terms of ethnicity. Tribal System of KPK and FATA is almost same and having same ethnic groups. People of Azad Jammu and Kashmir and Gilgit-Baltistan have different ethnicity. Punjab is the largest province of Pakistan based upon population. Chiniot is the 36<sup>th</sup> district of the Punjab, with a population of 965,124 individuals, according to Population Census of 1998, which is extrapolated for 2017, as ~1,316,620 individuals. It is one of the most historical cities of the country with history ranging from arrival of Alexander, the Great (326 BC). It has well diverse local population because it has been center of many civilizations during the course of time. Chiniot has three tehsils (an administrative subdivision), known as Bhowana, Chiniot and Lalian. Caste system, which is a part of Hindu religion social order, prevails in the Punjab. Most people after conversion to Islam kept their castes.

Surname and dominant caste varies from area to area throughout the subcontinent. Thus main caste in Bhowana is Sayal, in Chiniot is Sheikh and in Lalian is Lali. Many other castes, like Arain, Jatt, Malik, Rajpoot, etc., are common throughout Punjab. After partition of sub-continent in 1947, there occurred shuffling of Hindu and Muslim castes and ethnic groups, e.g., Pathan and Baloch, who are residing in district. In rural areas of district, many sub-castes of different minor castes are living, which prefer intra-caste marriages to preserve honor of their origin as a pure distinct entity.

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<sup>1</sup> Federally Administered Tribal Area

**Supplementary File 3:****INFORMED CONSENT FORM**

**PROJECT: Genetic Epidemiology, Risk Factors and Identification of Colour-blindness in Different Isonym Groups of District Chiniot, Punjab, Pakistan**

**PRINCIPAL INVESTIGATOR:** Prof. Dr. Muhammad Aslamkhan

**Co-INVESTIGATOR:** Muhammad Shoaib Akhtar

**INSTITUTION:** Department of Human Genetics & Molecular Biology  
University of Health Sciences Lahore

**INTRODUCTION:**

Purpose of the study is to find out the prevalence of colour-blindness in the district of Punjab, Chiniot, Pakistan.

تحقیق کا مقصد پنجاب (پاکستان) کے ضلع چنیوٹ میں کلر بلائنڈنس کی شرح معلوم کرنا ہے۔

**PROCEDURES:**

In this study we would fill a specially designed questionnaire to see association of various risk factors with colour-blindness.

تحقیق کے دوران کلر بلائنڈنس کی مختلف وجوہات سے ہم آہنگی جاننے کیلئے ایک مخصوص سوالنامہ پر کروایا جائے گا۔

**POSSIBLE RISKS:**

There is no risk involved in this study.

دوران تحقیق کوئی خطرہ لاحق نہیں۔

**FINANCIAL BENEFIT OR COMPENSATION:**

There is no financial benefit or compensation for your participation in this research.

تحقیق میں آپ کی شرکت کے بدلے کسی بھی طرح کا مالی تعاون ہرگز نہیں کیا جائے گا۔

**RIGHT OF REFUSAL TO PARTICIPATE AND WITHDRAWAL:**

Your participation in the study is completely voluntary and you may choose to stop participating at any time.

آپ کی شرکت رضا کارانہ ہوگی اور آپ کسی بھی موقع پر اپنی شرکت روک سکتے ہیں۔

**CONFIDENTIALITY:**

The information provided by you will remain confidential. Nobody except principal investigator will have an access to it. However, the data may be published in journal and elsewhere without giving your name or disclosing your identity.

آپ کی پہنچائی ہوئی معلومات کو مکمل طور پر خفیہ رکھا جائے گا اور پرنسپل انویسٹیگیٹر کے علاوہ کسی کو ان معلومات تک ہرگز رسائی حاصل نہیں ہوگی تاہم آپ کی شناخت ظاہر کیے بنا تحقیق کو چھاپا جاسکتا ہے۔

#### AVAILABLE SOURCES OF INFORMATION:

In case of any further questions, Principal Investigator, Prof. Dr. M. Aslamkhan from the Department of Human Genetics & Molecular Biology at University of Health Sciences may be contacted on following phone number 0300-4163225 ; (042) 9231304 – 09 ext 320.

مزید سوالات کے لیے پرنسپل انویسٹیگیٹر پروفیسر ڈاکٹر محمد اسلم خان ڈیپارٹمنٹ آف ہیومن جینیٹکس اینڈ مالیکیولر بائیولوجی یونیورسٹی آف ہیلتھ سائنسز لاجر سے رابطہ کریں۔

#### AUTHORIZATION

*I have read and understood this consent form, and I volunteer to participate in this research study. I am not waiving any of my legal rights by signing this form. My signature below indicates my consent.*

میں نے اس سوالنامے کو پڑھا اور سمجھ لیا ہے اور مطلوبہ تحقیق میں رضا کا اظہار پر شامل ہو رہا ہوں۔ میرے دستخط میری اجازت ظاہر کرتے ہیں۔

Name of Participant..... S/O, D/O.....

Signature/Thumb Impression.....Date:.....