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Original Article

Ophthalmology Section

A Clinical Study of Colour Vision Status of Female Relatives of Indian Males with Defective Colour Vision

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ABSTRACT

Introduction: Around 15% of females are carriers of congenital colour blindness. Variable colour vision status exists in these heterozygous carrier females due to various factors such as retinal mosaicism and the likelihood of the presence of an additional cone with different photosensitivity. These heterozygous carriers are considered to be tetrachromats.

Aim: To determine the colour vision status of female relatives of Indian colour blind males.

Materials and Methods: In this cross-sectional observational study, screening of males was done by Ishihara plates, and those with Colour Vision Defects (CVD) underwent a digital Farnsworth Munsell hue test (FM 100 test). First degree female relatives and a control group of normal females underwent the same tests. Statistical analysis was done using one-way ANOVA test, Tukey's test and unpaired t-test.

Results: Thirty one colour blind males accounted for 3.04% of the 1017 males screened. All participating female relatives, i.e., 31 obligatory carriers (23 mothers and 8 daughters) and 12 sisters, had normal test results on Ishihara plates as well as FM 100 test, barring one mother. FM error score above 70 is considered to be abnormal. The FM error scores in the obligatory carrier females ranged from 39 to 62, while that in sisters' group ranged from 36-67. This error score was lower in control group with range from 14 to 49. Although, all female relatives made errors within the normal range, they had statistically significant higher FM error scores than the controls.

Conclusion: Obligate carriers have normal colour vision on Ishihara plates. Higher error scores, albeit within normal limits, were seen on FM 100 test in obligate carriers than controls. Poor performances of genetic tetrachromats on the FM 100 test can be attributed to the fact that these tests are designed specifically for trichroamatic normal individuals, and thus, may not be sensitive enough to detect the superior colour discrimination abilities of tetrachromats. Detection of tetrachromacy will require customisation of colour vision tests as well as their availability to clinicians.

Keywords: Congenital colour blindness, Digital farnsworth munsel 100 hue test, Heterozygous obligatory carriers, Human tetrachromacy

INTRODUCTION

Congenital X-linked recessive defects are the most common type of Colour Vision Defects (CVDs) [1,2], with 15% of females being carriers of the trait [3]. Mothers and daughters of colour blind males are obligatory carriers [4] and hence, should have normal colour vision [5]. However, variable colour vision status has been reported in these heterozygous carrier females [3,6-8], due to a random inactivation of one of the two X chromosomes, leaving only one functioning X chromosome which may be normal or abnormal [9]. The retinas of heterozygous carriers have a mosaic of cone population having mutant alleles as well as normal alleles expressing four different types of cone pigments, i.e., one anomalous red or green pigment in addition to the normal red, green and blue cone pigments [3]. Hence, such heterozygous carriers possess four types of cones and may be considered to be tetrachromats with an additional cone pigment having a different photospectral sensitivity [3,10]. However, all genetic tetrachromats may not manifest tetrachromacy phenotypically, because of the random inactivation phenomenon.

There are previous reports of daughters and mothers of trichromatic anomalous males, who are able to discern many more hues than a normal person [11]. So far, only one tetrachromat female with supernormal colour vision has actually been identified to date [11]. This extremely rare detection may be attributed to the fact that the current, routinely used, colour vision tests are not designed to detect the possible superior colour discrimination in putative tetrachromatic carriers.

There are no previous studies on this aspect of colour vision in the Indian population; although, there are reports on the prevalence of colour blindness [12-14]. Against the above background, it was decided to study the colour vision status of female relatives of Indian colour blind males in a clinical setting and compare it with controls.

MATERIALS AND METHODS

A cross-sectional, observational study was conducted from April to July 2015, at a teaching hospital, after due approval by the institutional ethics committee. Healthy adult males, below 40 years of age, were enrolled from the college campus, hospital and community outreach centres. They were screened for colour blindness. Female relatives of colour blind males and a control group of normal female relatives of non colour blind males were also enrolled in the study. All participants were enrolled after obtaining informed consent.

Individuals taking drugs known to affect colour vision, such as tamoxifen, sildenafil, ethambutol, metronidazole, digoxin, and chloroquine were excluded from the study, as also those with a history of ocular or optic nerve disorders, or trauma.

Study Materials

Ishihara pseudo-isochromatic charts (38 plates), and a FM 100 test [15] were used to evaluate colour vision.

CVDs were detected with transformation plates, vanishing plates, and hidden digit plates of the Ishihara charts. Red (Protan) and

green (deutan) colour perception defects were differentiated by the diagnostic plates of the Ishihara charts [16].

The digital FM 100 test consists of four colour rows, each having 22 randomly arranged squares of different hues of one colour. Individuals were required to place the squares in a correct order of hue. The results were automatically reported as Total Error Scores (TES) by the computer. TESs above 70 indicated colour vision deficiency. The online FM 100 test was carried out on a Dell Inspiron N5010 laptop, with Microsoft Windows 7 version 6.1.7601 service pack 1 build 7601, using Intel®core™ i5 CPU, and graphics card ATI mobility Radeon HD 4650 1.00 GB. The resolution of the monitor display was 1366x768x60 Hertz with a 2.2 gamma value. The test was performed binocularly.

Study Protocol

All male participants were screened for CVD by Ishihara charts by the recommended standard procedure. Males with CVD underwent further testing by the online FM 100 test. Each individual performed the test thrice, as the test has a learning curve. The lowest of the three total error scores was noted.

Forty three female relatives (mothers/sisters/daughters) of the colour vision defective males were subsequently invited to participate in the study. They were divided into two groups; an obligate carriers group comprising of mothers and daughters, and a second group comprising of sisters of the men. The obligatory carrier group comprised of 31 females, while the sisters' group comprised of 12 females. A control group was formed of 90 healthy females, who were related to males with normal colour vision. All females underwent colour vision testing by both the above tests.

STATISTICAL ANALYSIS

Statistical analysis was done by the one-way ANOVA test, Tukey's test and the unpaired t-test, using the IBM SPSS statistics version 20.0 package. A p-value <0.05 denoted a level of significance.

RESULTS

Results of Males Groups

A total of 1017 healthy adult males between the ages of 18-40 years (mean age 26.68) were screened using the Ishihara test. Thirty one males were detected to have CVD i.e., an incidence of 3.04% (n=31). The age-wise distribution of results of the Ishihara test among participating males is shown in [Table/Fig-1]. Among the colour blind males, protanomaly was detected in 8 males (25.81%), while deuteranomaly was detected in 23 males (74.19%). The FM scores in 31 colour blind males ranged from 84 to 224. The average score of protanomalous males was 153.94 while that of deuteranomalous males was 167.5. The difference in the performance of protanomalous and deuteranomalous males on FM 100 test showed a p-value of 0.616 by unpaired t-test with higher error scores by deuteranomalous males.

Of the 133 females studied, 132 displayed normal colour vision on the Ishihara plates. One female, a mother from the obligatory carrier group, tested positive on Ishihara test manifesting deuteranomaly, and had an abnormal total error score of 164 on the FM 100 test. She was excluded from further analysis of the obligatory heterozygous carrier group, on the assumption that she was colour blind. Genetic testing is required to determine her genotype for colour blindness.

Age (in years)	Number of males screened (Total 1017)	Number of colour blind males detected on screening by Ishihara charts	Number of males with Protanomaly (Partial Red blind)	Number of males with Deuteranomaly (partial Green blind)
18-20	191	6	2	4
21-30	514	14	3	11
31-40	312	11	3	8
Total	1017	31	8 (25.81%)	23 (74.19%)

[Table/Fig-1]: Wise distribution of results of Ishihara tests of participating males.

Age group	Number of carrier females (n=30)	Mean score on FM 100 test	Standard deviation
10-20	8	46.50	5.66
41-50	12	52.67	5.12
51-60	6	55.50	5.43
61-70	4	55	6.83

[Table/Fig-2]: FM scores in obligatory carrier group (n=30).

Age group	Number of sisters (n=12)	Mean score on FM 100 test	Standard deviation
10-20	5	49.60	10.85
21-30	5	38.40	1.82
31-40	2	55	4.24

[Table/Fig-3]: FM scores in sisters' group.

Age group (in years)	Number of control females (n=90)	Mean score on FM 100 test	Standard deviation
11-20	24	24.92	5.96
41-50	36	33.53	5.26
51-60	18	36.11	5.73
61-70	12	40.67	5.05

[Table/Fig-4]: FM scores in control females (n=90).

Results of Female Groups

The final 'obligatory carriers' group comprised of 30 females i.e., 22 mothers and 8 daughters, while the 'sisters' group comprised of 12 females. Ninety normal females, age matched with the carrier group, acted as controls. The FM error scores in the three groups of female participants were as shown in [Table/Fig-2-4].

The FM scores among carrier females ranged from 39 to 62 (mean=51.90). There were 8 carriers of protanomaly with average score of 48.63 while there were 22 deuteranomaly carriers with average score of 53.09. Difference between mean scores of protanomaly carriers and deuteranomaly carriers had a p-value of 0.085 by unpaired t-test with worse scores obtained in deuteranomalous carriers. The FM scores in the sisters' group ranged from 36 to 67 (mean=45.83). There were four sisters of protanomlous males with average score of 46.5, while there were eight sisters of deuteranomalous males with average score of 45.5. The mean scores of sisters of protanomalous males were worse than those of sisters of deuteranomalous males (p=0.87 by unpaired t-test). The scores among controls ranged from 14 to 49 (mean=32.7).

Sr. No	Group	Age group with least error scores	p-value by one-way ANOVA test (Tukey's test)
1.	Obligatory carriers	10 years to 20 years	0.021
2.	Sisters	21 years to 30 years	0.042
3.	Controls	10 years to 20 years	<0.01

[Table/Fig-5]: Age-wise comparison of performance on FM 100 test in different female groups.

Both obligatory carriers, as well as sisters revealed poorer performances on FM, when compared to the controls by post hoc ANOVA test (Tukey's test) (p<0.01). Amongst the female relatives, scores of carriers were worse than those of the sisters (p=0.057 by Tukey's test). Younger females in all groups performed better than older females on the FM 100 test [Table/Fig-5].

DISCUSSION

A total of 1150 individuals (1017 males, 133 females) participated in this study.

Colour Vision in Males

The Ishihara test, (95% to 98% sensitivity for red/green CVDs) [17], was used for the initial screening of 1017 males (mean age 26.68 years). The incidence of colour blindness was found to be 3.048% (31 out of 1017). This is comparable with the prevalence rate of 3.16% to 3.7% reported by various other studies conducted in India [12,13]. However, the lower prevalence reported in the present study as compared to the incidence of 8% reported worldwide [14] can be attributed to ethnicity differences.

The ratio of protans to deutans was noted to be 1:3. Various studies in past have reported this ratio to range from 1:2 to 1:10 [13,14].

All males with CVD were next administered the online FM 100 test. Results of FM 100 test performed digitally were equivalent to those obtained on the manual test [18]. The use of a laptop for conducting the digital FM 100 test was found to be convenient at all locations.

Colour Vision in Heterozygous Obligate Carrier Females

None of the 30 obligate carriers in our study committed any errors on the Ishihara plates. Similar reports of normal response on Ishihara plates has been noted in other studies as well [7,19]; although, a few studies [3,6], have reported higher error scores on Ishihara plates by carrier females. These differing reports of variable colour vision status of heterozygous carrier may be explained by the known variability in expression of the heterozygous trait as discussed earlier.

In the present study, obligatory heterozygous carriers had a poorer performance on FM 100 test than their age matched controls (p-value <0.01). This is in agreement with the results of studies done by Krill AE and Schneiderman A [7] and Verriest G [20]; although, some authors [3,6] have reported in significant differences in performance on FM 100 test between carriers and controls .

In all the above mentioned studies, a manual FM 100 test was used, while in the present study, a digital version of the test was used. Variability in the scores of FM has been noted by Murphy RA [21] and is due to a slight learning curve for performing the test. A minimal training is said to result in 30% improvement in the TES of FM 100 test [22]. A similar improvement in results was observed by us with repetition of the test, and, therefore, the lowest error score out of three attempts was noted for each participant.

Heterozygous Carriers and Tetrachromacy

Jameson KA et al., has reported that individuals with tetrachromatic genotype perform poorly on the FM 100 test [23,24]. They opined that the standard FM 100 test which is currently available lacks the capability to detect discrimination of additional hues; an ability which the tetrachromats may display [24]. These tests are designed for use in trichromatic normal individuals and may thus give higher error scores as perception of hues is different (superior) in heterozygous female carriers. We have noted a similar poor performance by members of the carrier group as compared to the controls on FM 100 test. Some of these heterozygous carriers could possibly be phenotypical tetrachromats, which cannot be evaluated with the current FM tests. The discovery of the first human tetrachromat was possible because of the use of a customised colour vision test (Multidimensional scaling) [11]. It is possible that with the advent of newer and more sophisticated colour vision tests, many of our female obligatory carriers would also exhibit superior colour discrimination. Genetic testing and use of sophisticated customised colour vision tests were both beyond the scope of our study.

Performance of Deutan and Protan Carriers

Our study revealed greater error scores amongst deuteranomaly carriers compared to protan carriers. Hood SM et al., studied chromatic discrimination along red green axis among carriers of red green colour vision deficiency, and found it to be maximally affected in deutan carriers [8]. This difference can be explained by the fact that the ratio of red (L) and green (M) cones has an impact on the colour discrimination. The closer this ratio is to 1:1, the better the colour discrimination [8]. Generally, it is seen that deutan carriers have a poorer colour discrimination due to the presence of a skewed L:M cone ratio [8].

A difference in the results of the FM 100 test by deutan carriers and protan carriers was not statistically significant in our study (p=0.085).

The Impact of age on the Performance of the Online FM 100 Test

The impact of age on the performance of the online FM 100 test amongst female subjects could be inferred from the fact that younger subjects performed better in the obligatory carrier group (p=0.021), the sisters' group (p=0.042) as well as the control group (p=<0.01). Similar observations of younger individuals performing better on FM 100 test, have been made by several other workers [7,25]. Pupil size, background illumination level, iris colour and macular pigment density are considered to be responsible for the age-related increase in FM 100 hue error scores [26].

Performance of the Sisters Group

There were four sisters of protanomalous males, and eight sisters of deuteranomalous males. The group comprising sisters was considered to be a likely mixture of carriers and normal females. It is not possible to state the carrier status of the sisters without definitive genetic analysis. All the sisters tested normal on Ishihara plates, and had TES <70 on FM testing. Like the obligate carriers, they too made a greater number of errors compared to the controls (p=<0.01). It is possible that, in the present study, the sisters group had a disproportionately higher number of sisters with a carrier status than normal sisters. Genetic testing would be able to resolve this issue.

The study found poor colour discrimination ability amongst heterozygous carriers as compared to the controls. Whether the carriers are tetrachromats or not, could not be determined by the conventional methodology used in the present study, the use of the digital FM 100 test not with standing. Detection of tertachromacy with superior colour vision discrimination requires customisation of the colour vision test such as multidimensional scaling which was used to find out first tetrachromat female [11]. Tetrachromacy is likely to be detected to a greater extent, as and when newer testing techniques become available at in the clinical setup.

LIMITATION

Small number of obligatory carrier females studied, lack of genetic testing for tetrachromacy and non availability of sophisticated colour vision tests (anomaloscope).

CONCLUSION

Obligate carriers of colour blindness have normal colour vision on Ishihara plates. Higher error scores, albeit within normal limits, were seen on FM 100 test in obligate carriers than controls. These obligatory carriers are putative tetrachromats. Poor performance of genetic tetrachromats on the FM 100 test can be attributed to the fact that these tests are designed specifically for trichromatic normal individuals, and thus, may not be sensitive enough to detect the superior colour discrimination abilities of tetracromats. Detection of tetrachromacy will require customisation of colour vision tests as well as their availability to clinicians.

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