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Morphometric Analysis Of Chromosomes Among Meos And Sunni Muslims Of Haryana

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Abstract

Among human beings inbreeding is usually uncommon because of social conventions and laws although in small isolated populations it does occur, mainly through mating between relatives. The most common type of close inbreeding is between first cousins. The effect is always an increase in frequency of genotypes that are homozygous for rare, usually, harmful recessive trait. Cytogenetical investigation was planned on Meos and Sunni Muslims of Haryana because instances of close inbreeding were observed among them. Blood samples of 56 individuals, both male and female, belonging to Meos and Sunni Muslims were taken. Short term lymphocyte cultures were set up according to technique of Moorhead et al. (1960). As many as, 100 well spread metaphases were selected from the slide of each individual for chromosomal aberrations, karyotype preparation. Morphometric analysis of chromosomes with respect to percentage relative length, arm ratio and centromeric index were found to vary in Meos and Sunni Muslims. Results of "t-test" of percentage relative length differences of individual chromosomes were found to be significant for chromosomes 2, 6, 8, 12, 18 and X.

Keywords: Inbreeding, Morphometric analysis, Karyotype preparation, Arm ratio.

Introduction

Although classification of human chromosomes into seven groups (A-G) was a significant achievement, most of the extra or structurally altered chromosomes could not be identified. Levan and Hsu (1959) were the first to demonstrate that there could be considerable difference in length between the two homologous chromosomes with in the same cell. Chromosome complements with minor morphological deviations having no phenotypic disadvantage are maintained frequently in a population. Selective forces operating on them are, however, not precisely known.

Conventional staining techniques have shown that secondary constriction regions of short arms of acrocentrics, chromosomes, 1, 16, and Y chromosome show variation in size and/or morphology (Patau, 1961; Lubs and Ruddle, 1970). The major breakthrough in chromosomal identification occurred after Casperson et al. (1968) demonstrated that each chromosome has its own anatomy by virtue of its banding pattern. The new chromosomal banding techniques have not only allowed a more specific and detailed characterization of already

known heteromorphism but have also revealed new heteromorphism (Schnedl, 1971). Chromosomes deviating from normal morphology were referred to as 'Variant' in Paris conference (1971) recommended the term heteromorphic to the chromosomes with variable band. Herteromorphism has been attributed to herterochormatic component of chromosomal complement which is considered to be genetically inert.

Various possible functions of heterochromatin like organization of the chromosome systems, attracting homologues at meiosis, facilitating evolutionary changes, providing raw material for new genes, acting as gene spacers and loci for recombination and protection of euchromatin from mutagens have been suggested (Hsu, 1975). Short arm regions of D and G group acrocentric chromosomes were among the earliest recognized heteromorphisms in the human karyotype. Due to presence of satellites in all the ten chromosomes of D and G group, they have the property to vary in size and degree of heteropycnosis (Engmann, 1967). Bahr and Golomb (1971) observed about 10% frequency of giant satellite to be inherited in highly inbred Amish community. Higher frequency of enlargement of short arm and satellite region in chromosome 15 than chromosome 13 and 14 was revealed by banding techniques. This frequency, however, was approximately equal in chromosome 21 and 22 (Ferguson – Smith, 1974; Nielsen et al., 1974; Tharapel and Summit, 1976). Various population surveys have revealed a low frequency of absence of short arm of D and G group acrocentrics (Mikelsaar et al., 1973, 1975; Bochkov et al., 1974; Hamerton et al., 1975; Walzer and Gerald, 1977).

Materials and Methods

For cytogenetic studies 28 individuals from each caste (Meos and Sunni Muslims) were selected. About 10 ml blood was taken from each individual in heparin coated green top tube by vein puncture in the arm. Then, lymphocyte culture was set up for cytogenetic studies. Short term lymphocyte cultures were setup (Moorhead et al., 1960). About 5ml of medium (RPMI-1640) was taken into different culture vials. 0.5-0.8ml of heparinised whole blood was delivered to each culture vial and capped tightly. The contents of the culture bottle were thoroughly mixed by repeatedly tapping the bottom. The cultures were kept in an incubator in a slanting position at 37°C. Cultures were incubated for 72 hours. Two hours prior to harvesting 0.02ml (10µg/ml) colchicine solution was added to each culture vial. The culture vials were gently shaken and incubated for additional 50-60 minutes at 37°C. After this the tubes were centrifuged at 800 rpm for 8 minutes. The supernatant was discarded leaving the cell button in the tube. The cell button was resuspended in 5ml of hypotonic solution (0.075M KCl) already warmed at 37°C. These tubes were incubated for 10-15 minutes at 37°C followed by centrifugation at 800 rpm for 8 minutes. After the removal of supernatant freshly prepared fixative (3:1, Methanol: glacial acetic acid) was added slowly to the cell button. The supernatant was again discarded, pellet was resuspended in fixative and tubes were again centrifuged. This process was repeated 3-4 times until the pellet turned white and the fixative appeared clear. After the final centrifugation, the cells were suspended in 0.5 to 1ml of fresh fixative to form a slightly milky suspension. Then 2-3 drops of cell suspension were dropped evenly from a

height of about 2 feet on a wet, cold, grease free glass slide. The slides were kept in a slanting position and allowed to dry at room temperature. Then the slides were coded and studied under Trinocular Research Microscope. The slides were subjected to GTG banding (Seabright, 1971). Photo micrographs of some selected metaphases were also taken for record to study various cytogenetic parameters viz. relative length of chromosome, centromeric index, arm ratio of various chromosomes. Identification of the chromosomes was done according to the ISCN report (ISCN, 1985). For morphological classification of the chromosome the criteria and terminology proposed by Passarge (1974) was followed.

Results

During the present investigation, the morphometric analysis of chromosomes was accomplished among Meos and Sunni Muslims of Haryana. The representative karyotypes of male and female individuals belonging to and Meos & Sunni Muslims are given in figs. 1 and 2. G-banding was also accomplished for karyotyping. (Figs. 3 and 4).

The details of morphometric analysis with regard to percentage relative length, arm ratio and centromeric index of each chromosome has been presented in tables 1 and 2 for Meos and Sunni Muslims respectively.

Table 1: Morphometric data of Somatic Karyotype of Meos

Chromosome Pair no.	Relative length %				Arm Ratio				Centromere Index				Chromosome Type
	Mean	S.D.±	S.E.	C.V.	Mean	S.D.±	S.E.	C.V.	Mean	S.D.±	S.E.	C.V.	
1.	8.878	0.789	0.149	8.887	1.045	0.149	0.028	14.528	48.908	2.862	0.541	5.852	Metacentric
2.	7.171	0.741	0.140	10.333	1.587	0.299	0.057	18.841	38.659	3.025	0.572	7.825	Submetacentric
3.	6.659	0.648	0.122	9.731	1.200	0.188	0.022	9.833	45.444	2.562	0.484	5.638	Metacentric
4.	6.649	0.642	0.121	9.656	2.746	0.342	0.065	12.454	26.268	2.927	0.553	11.145	Submetacentric
5.	6.329	0.611	0.115	9.654	3.240	0.487	0.092	15.031	23.583	3.436	0.649	14.570	Submetacentric
6.	5.952	0.592	0.112	9.946	1.717	0.307	0.058	17.880	36.807	2.125	0.402	5.773	Metacentric
7.	5.486	0.554	0.105	10.098	1.598	0.354	0.067	18.515	38.480	3.025	0.572	7.861	Metacentric
8.	5.137	0.521	0.098	10.142	1.912	0.282	0.053	14.749	33.962	2.455	0.464	7.229	Metacentric
9.	4.488	0.509	0.096	11.341	2.046	0.294	0.056	14.370	33.339	3.565	0.674	10.693	Submetacentric
10.	4.255	0.448	0.085	10.529	2.048	0.322	0.061	15.723	32.829	3.112	0.588	9.479	Submetacentric
11.	4.109	0.442	0.084	10.757	2.164	0.329	0.062	15.203	31.603	2.678	0.506	8.474	Metacentric
12.	3.896	0.417	0.079	10.703	2.526	0.349	0.066	13.816	28.538	2.085	0.394	7.352	Submetacentric
13.	3.693	0.428	0.081	11.589									Acrocentric
14.	3.208	0.397	0.075	12.375									Acrocentric
15.	3.162	0.413	0.078	13.061									Acrocentric
16.	3.140	0.358	0.068	11.401	1.746	0.278	0.053	15.922	36.419	3.485	0.659	9.569	Metacentric

17.	2.781	0.348	0.066	12.513	1.977	0.242	0.046	12.241	33.593	3.576	0.676	10.645	Submetacentric
18.	2.633	0.343	0.065	13.027	2.586	0.287	0.054	11.098	27.888	3.872	0.732	13.884	Submetacentric
19.	2.268	0.317	0.060	13.977	1.256	0.158	0.030	12.580	44.334	4.087	0.772	9.219	Metacentric
20.	1.925	0.298	0.056	15.481	1.309	0.098	0.019	7.487	44.303	2.171	0.410	5.014	Metacentric
21.	1.745	0.282	0.053	16.160									Acrocentric
22.	1.376	0.293	0.055	21.294									Acrocentric
X	5.855	0.599	0.113	10.231	1.581	0.204	0.039	12.903	38.741	3.195	0.604	8.247	Metacentric
Y	1.569	0.392	0.074	24.984									Acrocentric

Table 2: Morphometric Data of Somatic Karyotype of Sunni Muslims

Chromosonal Pair no.	Relative length %				Arm Ratio				Centromere Index				Chromosome Type
	Mean	S.D.±	S.E.	C.V.	Mean	S.D.±	S.E.	C.V.	Mean	S.D.±	S.E.	C.V.	
1.	8.577	0.721	0.136	8.406	1.040	0.129	0.024	12.404	48.866	2.983	0.564	6.104	Metacentric
2.	7.505	0.685	0.129	9.127	1.501	0.287	0.054	19.121	39.971	3.122	0.590	7.811	Submetacentric
3.	6.635	0.695	0.131	10.475	1.202	0.127	0.024	10.566	45.409	2.467	0.466	5.433	Metacentric
4.	6.581	0.678	0.128	10.302	2.834	0.326	0.062	11.503	26.082	3.246	0.613	12.445	Submetacentric
5.	6.037	0.602	0.114	9.972	2.343	0.389	0.074	16.603	29.909	2.801	0.529	9.365	Submetacentric
6.	5.549	0.562	0.106	10.128	1.803	0.292	0.055	16.195	35.672	2.805	0.530	7.863	Metacentric
7.	5.128	0.537	0.101	10.472	2.022	0.362	0.068	17.903	33.092	2.980	0.563	9.005	Metacentric
8.	4.819	0.528	0.100	10.975	1.858	0.259	0.049	13.940	34.311	2.222	0.420	6.473	Metacentric
9.	4.549	0.488	0.092	10.728	2.314	0.285	0.054	12.316	30.176	2.982	0.564	9.882	Submetacentric
10.	4.384	0.503	0.095	11.474	2.007	0.310	0.059	15.446	33.250	3.470	0.656	10.436	Submetacentric
11.	4.133	0.469	0.089	11.348	2.275	0.341	0.064	14.989	30.526	2.435	0.460	7.977	Metacentric
12.	3.655	0.458	0.087	12.531	1.754	0.288	0.054	16.420	36.969	2.646	0.500	7.157	Submetacentric
13.	3.633	0.433	0.082	11.919									Acrocentric
14.	3.329	0.401	0.076	12.046									Acrocentric
15.	3.263	0.368	0.070	11.278									Acrocentric
16.	3.241	0.352	0.067	10.861	1.439	.248	.047	17.234	41.006	3.188	0.602	7.774	Metacentric
17.	2.980	0.379	0.072	12.718	2.513	.309	.058	.12.296	28.467	3.022	0.571	10.616	Submetacentric
18.	2.839	0.352	0.067	12.399	2.346	.253	.048	10.784	29.885	3.675	0.695	12.297	Submetacentric
19.	2.306	0.333	0.063	14.441	1.510	.177	.033	11.722	39.837	3.127	0.591	7.849	Metacentric
20.	2.012	0.302	0.057	15.010	1.229	.084	.016	6.835	44.864	2.925	0.553	6.520	Metacentric
21.	1.762	0.262	0.050	14.869									Acrocentric
22.	1.346	0.230	0.043	17.088									Acrocentric
X	5.421	0.617	0.117	11.382	2.339	.312	.059	13.339	30.00	2.765	0.523	9.217	Metacentric
Y	1.691	0.298	0.056	21.119									Acrocentric

Table 3: Results of 't-tests' of Percentage Relative Length of the Chromosomes of Meos and Sunni Muslims of Haryana

Chromosome No.	t value	Chromosome No.	t value
1.	1.4902	13	0.5215
2.	2.2758*	14	1.1347
3.	0.1337	15	0.9662
4.	0.3854	16	1.0645
5.	1.8014	17	1.9643
6.	2.6125*	18	2.2179*
7.	1.3395	19	0.4374
8.	2.2685*	20	1.0851
9.	0.4578	21	0.2337
10.	1.0134	22	0.2634
11.	0.1971	X	2.6706*
12.	2.0589*	Y	1.6979

* Significant ($p<0.05$)

Morphometric analysis of the chromosomes with respect to relative length percentage, arm ratio and centromeric index were found to vary among the Meos and Sunni Muslims of Haryana. Among the Meos, the higher values for percentage relative length were revealed for the chromosomes 1, 3, 4, 5, 6, 7, 8, 12, 13, 22, X, Y and the lower values for chromosomes 2, 9, 10, 11, 14, 15, 16, 17, 18, 19, 20, 21 and vice–versa for Sunni Muslims. Among the Meos the higher values for arm ratio were revealed for the chromosomes 1, 2, 5, 8, 10, 12, 16, 18, 20 and lower values for chromosomes 3, 4, 6, 7, 9, 11, 17, 19, X and vice-versa for Sunni Muslims. Among the Meos higher values for centromeric index were revealed for chromosomes 1, 3, 4, 6, 7, 9, 11, 17, 19, X and the lower values for chromosomes 2, 5, 8, 10, 12, 16, 18, 20 and Y. Results of the "t-test" of percentage relative length of the various chromosomes are given in table 3. **(30.)** The results were found to be statistically

significant for chromosome 2, 6, 8, 12, 18 and X. For the other chromosomes the results were statistically non-significant.

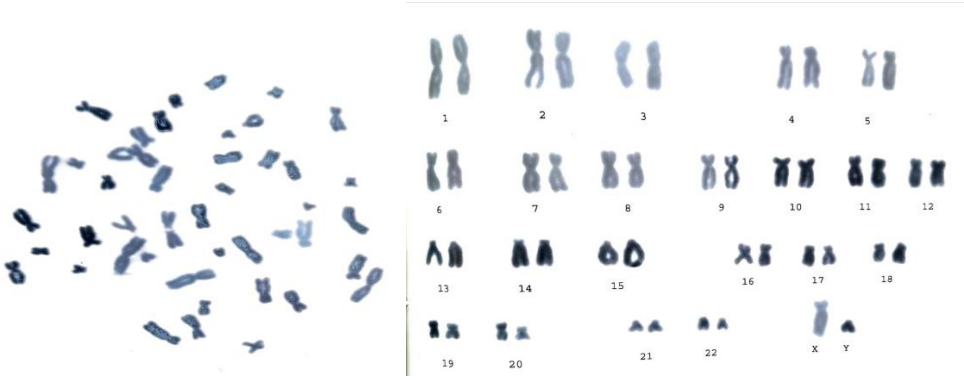

Fig 1: Mitotic Metaphase and its Karyotype in a male individual.

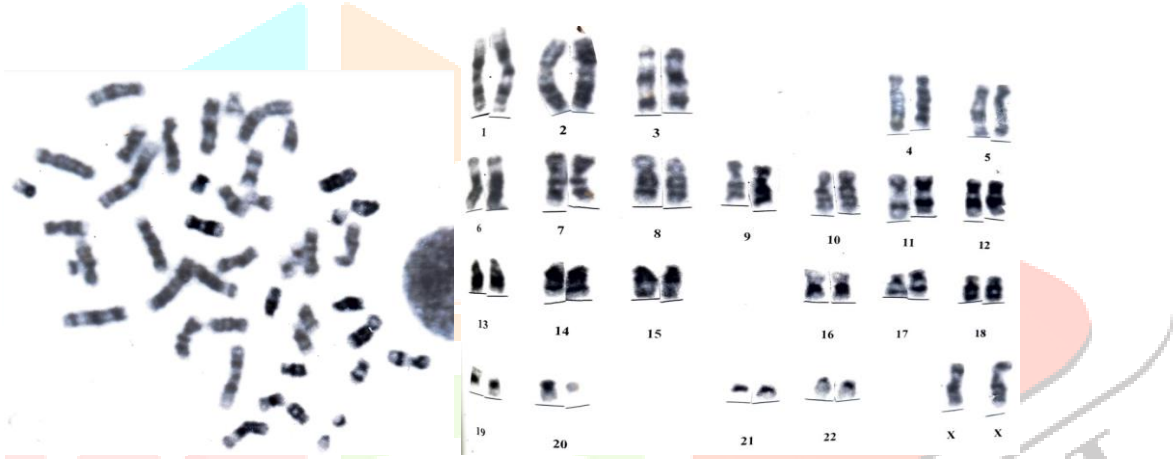

Fig 2: G-Banded Metaphase and its Karyotype in a female individual.

Discussion

The basic classification of human metaphase chromosomes was derived from conferences in Denver (Denver Conference, 1960), London (London Conference, 1963), Chicago (Chicago Conference, 1966), Paris (Paris Conference, 1971). Three parameters were found to be important for basic morphological characterization of a chromosome i.e. relative length, chromosome arm ratio and centromere index. An important morphological feature of acrocentric chromosomes (D group and G group) was also noted i.e. satellites, although they were not always visible.

The detailed morphometric analysis of human Karyotype as by Passarge (1974) have been followed for describing and comparing the chromosomes in the present investigation.

Group A.

Chromosome 1 is the largest metacentric chromosome of the karyotype. The chromosome arm ratio is 1.1 and the centromeric index 48-49 (Passarge 1974). In the present investigation Meos showed 1.045 arm ratio and 48.908 centromeric index (Table 1), whereas Sunni Muslims showed arm ratio of 1.040 and centromeric index of 48.866 (Table 2). These values fit well with in the standard range. Chromosome 2 is the largest

submetacentric chromosome with an arm index of 1.5-1.6 and centromeric index of 38-40. In the present investigation, Meos exhibited 1.587 arm ratio and 38.659 centromeric index and Sunni Muslims showed arm ratio of 1.501 and centromeric index of 39.971. These values fit well with in the range. Chromosome 3 is the second largest metacentric chromosome, with an arm ratio of 1.2 and centromeric index 45.46. This chromosome is reported to be about 20% shorter than chromosome 1. In the present investigation, Meos showed arm ratio of 1.2 and centromere index of 45.444 and Sunni Muslims showed arm ratio of 1.202 and centromere index of 45.409. The observed values are very close to the established range.

Group B.

Chromosomes 4-5 cannot be differentiated on morphological grounds alone. They are large distinctly submetacentric chromosomes. Their arm index is 2.6-3.2 and centromere indeed is 24-30. No chromosome of C group has such a low centromere index and they can hardly be confused with this group. Patau (1965) considers length measurements not to be useful in differentiating two pairs. Arm ratio value and centromeric index values for chromosome 4 in Meos (2.746, 26.268) and Sunni Muslims (2.834, 26.082) fit well within range. For chromosome 5 the arm ratio value in Meos (3.240) is slightly higher than reported range while centromeric index (23.583) is slightly lower than reported range. In Sunni Muslims the arm ratio value (2.343) is lower than reported range while centromeric index (29.909) is within the range.

Group C.

No individual chromosome of the group C and X can be easily distinguished. According to London convention (London Conference, 1963) pairs no. 6,7,8 and 11 are relatively metacentric with a centromeric index of about 35-40, whereas pairs no. 9, 10 and 12 are relatively submetacentric with a lower centromeric index of 27-35. These differences can be used to pair homologous chromosomes. Some authors think these efforts are useless (Patau, 1965). While others (Turpin and Lejeune, 1965) feel that a morphological differentiation within C group is possible. Chromosome 6 is the largest chromosome followed by X chromosome. In Meos the centromeric index value for chromosomes 6, 7, 8, 9, 10, 11 and 12 are 36.807, 38.480, 33.962, 33.339, 32.829, 31.603 and 28.358 respectively. Most of the values fit well in the range except chromosomes 8 and 11, which have lower values than the reported range. In Sunni Muslims, the centromeric index value for chromosomes 6 to 12 are 35.672, 33.092, 34.311, 30.176, 33.250, 30.526, 36.969 respectively. These values differ from reported range for chromosome 7, 8, 11 and 12. The X chromosome is relatively metacentric but has a wide range of centromeric index i.e. 26-38. In the present investigation, centromeric index for Meos is 38.741 and for Sunni Muslims is 30.000. The value for Meos slightly deviated on upper extreme and for Sunni Muslims it corroborated well with the range.

Group D.

Chromosome 13-15 are easily recognized as a group of large acrocentric chromosomes. The centromeric index is lowest in these chromosomes. These three pairs carry satellites which, probably for technical reasons and also because of individual variation in size, are rarely visible all together in a single cell. The D group chromosomes differ slightly in length (up to 10%) which will allow their arrangement in descending order of

length. In the present investigation, satellites are visible only in few chromosomes, so centromeric index was not calculated. Chromosomes were arranged on the basis of size in descending order.

Group E.

Chromosome 16-18 of this group are fairly short and have a median or sub median centromere. Chromosome 16 is metacentric with an arm index of 1.4-1.8 and a centromeric index of about 40. In Meos the arm index and centromere index for this chromosome were 1.746 and 36.419. The centromeric index value is less but arm index is in agreement with reported range. In Sunni Muslims, for chromosome 16, the arm ratio (1.439) and centromeric index (41.006) are close to reported range. Chromosome 17 is relatively submetacentric with arm index 1.8-3.1 and centromeric index 31 (23-36) indicating proximal position of centromere. The arm ratio and centromeric index values observed in Meos (1.977, 33.593) and in Sunni Muslims (2.513, 28.467) fit well within the reported range. Chromosome 18 is 5-10% shorter than chromosome 17 and it has arm index of 2.4-4.2 and centromere index of 26 (21-29). The arm ratio and centromere index value observed in Meos (2.586, 27.888) fits well within the reported range but in Sunni Muslims the arm ratio (2.346) is slightly lower and centromere index (29.885) is slightly higher than reported range.

Group F.

Chromosome 19 and 20 are small metacentric chromosome with arm index of 1.2-1.9 and Centromeric index approximately 40 (34-46). The arm ratio and centromere index for these chromosomes respectively in Meos (1.256, 1.309; 44.334, 44.303) and in Sunni Muslims (1.510, 1.229; 39.837, 44.864) fit well with in the range.

Group G.

Chromosomes 21-22 are very short acrocentric chromosomes with satellites at the end of short arm, though these usually are not all apparent in cell. The smaller one is new designated as No. 21. In the present investigation satellites are observed only in few chromosomes both in Meos and Sunni Muslims.

In the cytogenetic studies the human chromosomes occupy the top position, despite this, these are not characterized on morphometric basis alone (Weaver and Hedrick, 1992). The main difficulty is due to the presence of extensive inter-and intra-genotypic variability both with in and between humans, probably due to both random as well as non-random, breeding strategies, which human follows. This variability has also been reflected in the ISCN (1985) report as well as in a study by Kler ,1994 in five endogamous groups of Haryana and also during the present work in which the chromosomes of two ethnic groups (Meos and Sunni Muslims) were studied. These groups frequently differ among themselves with respect to percentage relative length of the chromosomes. Moreover, each group is specifically characterized by having a particular chromosome or chromosome group exhibiting maximum or minimum values of the percentage relative length, arm ratio and centromeric index. This shows that each of the two ethnic groups of Haryana is characterized by definite pattern of somatic chromosomes.

A, C, D, E, F and Y group chromosomes in Sunni Muslims (7.572, 4.888, 3.408, 3.020, 2.159, 1.691) exhibit the higher values of percentage relative length as compared to those of Meos (7.569, 4.760, 3.354, 2.851,

2.097, 1.560). On the other hand, percentage relative length value of B, G and X group chromosome in Meos (6.489, 1.561, 5.855) is higher than in Sunni Muslims (6.309, 1.554, 5.421). The arm ratio values for A and B group chromosome was higher in Meos (1.277, 2.993) than in Sunni Muslims (1.248, 2.589). But the arm ratio values for C, E, F and X group chromosomes were higher in Sunni Muslims (2.005, 2.105, 1.369, 2.339) than in Meos (2.002, 2.103, 1.283, 1.581). The centromeric index values were higher for C, F and X group chromosomes in Meos (33.625, 44.319, 38.741) than observed for these chromosomes in Sunni Muslims (33.428, 42.351, 30.00) whereas the centromeric index values for A, B, and E group chromosomes were higher in Sunni Muslims (44.749, 27.996, 33.119) than those observed in Meos (44.337, 24.925, 32.633). Why each of the two group exhibit the chromosome group specificity, remains an open question.

Though each of two ethnic groups exhibited chromosome specificities, "t-test" on individual chromosome with respect to percentage relative length does not reveal these intrinsic specificities, except chromosomes 2, 6, 8, 12, 18 and X (Table 3) which show significant differences. Similarly, no chromosome group specificity was observed for the two ethnic groups as all the chromosome group (A, B, C, D, E, F and G) exhibit non-significant "t-test" values i.e. 0.0157, 1.0631, 0.5020, 0.4070, 0.3551, 0.3254, 0.2678 respectively for each group.

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