GENETIC ANALYSES OF THE MAJOR TRIBES OF ABBOTTABAD AND MANSEHRA DISTRICTS THROUGH DENTAL MORPHOLOGY AND DNA ANALYSES

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DEPARTMENT OF GENETICS
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By

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بسم الله الرحمن الرحيم

AL QURAN

"O Mankind, we created you from a single pair of a male and a female, and made you in to tribes and nations so that you may know each other (not that you despise each other). Verily, the most honored of you in the sight of Allah is he who is most righteous of you, Surely Allah is All-Knowing, All-Aware."

(*Al-Hujurat*, 49: 13)

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Nazia Akbar Khan

Dedication

To My

Grandfather; The late Baba

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ABBREVIATIONS

AWAm1	Awans collection from Mansehra by BE Hemphill
AWAm2	Awans collection from Mansehra by the author
ChlMRG	Early Chalcolithic Period collection from the archeological site of Mehrgarh (*c.* 4500 BC)
CHU	Living tribal Chenchus from central Andhra Pradesh
DJR	Djarkutan Period collection from the archeological site of Djarkutan (2000-1800 BC)
GPD	Low-status Dravidian-speaking Gompadhompti Madigas from Southern Andhra Pradesh
GUJm2	Gujars collection from Mansehra by the author
HAR	Mature Period collection from the archeological site of Harappa (c. 2300-1800 BC)
INM	Late Jorwe Period collection from the archeological site of Inamgaon (*c.* 1400 BC)
KARa	Karlals collection from Abbottabad by the author
KHO	Khoward from Chitral City, Chitral District
KUZ	Kuzali Period collection from the site of Djarkutan (1800-1650 BC)
MDK	Living inhabitants of the village of Madak Lasht, Chitral District
MDA	Living Madia Gond tribals from Eastern Maharashtra
MDS	Multidimensional Scaling
MHR	Living Indo-Aryan-speaking low-status Mahars from Western Maharashtra
MRT	Living Indo-Aryan-speaking high-status Marathas from Western Maharashtra
MOL	Molali Period collection from the site of Djarkutan (1650-1500 BC)
NeoMRG	Aceramic Neolithic Period collection from the site of Mehrgarh (*c.* 6000 BC)

PNT	High-status Dravidian-speaking Pakanati Reddis from Southern Andhra Pradesh
PCO	Principal Coordinates Analysis
SAP	Sapalli Period collection from the site of Sapalli tepe (c. 2200-2000 BC)
SKH	Iron Age collection from the site of Sarai Khola (c. 200 BC)
SWT	Living Swatis collection from Dhodial and Baffa by BE Hemphill
SYDm2	Living Syeds collection from Mansehra by the author
TANm2	Tanolis collection from Mansehra by the author
TMG	Late Bronze/Early Iron Age collection from the site of Timargarha (1400-800 BC)
WAKg	Living Wakhis from Gulmit, Gilgit-Baltistan
WAKs	Living Wakhis from Sost, Gilgit-Baltistan
MtDNA	Mitochondrial DNA
CRS	Cambridge Reference Sequence
HV	Hyper variable
HVS	Hyper variable Sequence
HVSI	Hyper variable Sequence I
HVSII	Hyper variable Sequence II
Ab & AA	Abbassis from Abbottabad
A, Aw & AwM	Awans samples from Mansehra
Guj & G	Gujars from Mansehra
Jad & J	Jadoons from Abbottabad
Kar & K	Karlals from Abbottabad
Syd & S	Syeds from Mansehra
Tan & T	Tanolis from Mansehra

ABSTRACT

The Hazara Division of Khyber Pakhtunkhwa Province-Pakistan possesses an interesting combination of social integrity in the ethnic diversity of the region. People of the area can broadly be divided into the ancient Dards, Kushans and recently arrived people of Pathan dynasties. A number of references are available about the history and culture of the area but the general biology and phylogenetic relationship of people of the area based upon sound scientific grounds are still lacking. This dissertation provides first-hand information with reference to some of the important tribes of the area analyzed through variation in dental morphology, mitochondrial DNA sequences and haplotypic diversity in the people of central districts viz. Abbottabad and Mansehra of Hazara Division. The study was conducted from October 2010 to March 2014. Seven tribes viz. Abbassi, Awan, Gujar, Jadoon, Karlal, Syed and Tanoli were included in this study. Collection of dental casts, saliva samples for DNA isolation, optimization of PCR conditions, gene clean protocols and data analyses etc. were done in the Human Genetics Lab, Department of Genetics Hazara University. All the selected tribes were analyzed for Hyper Variable Sequences of mitochondrial DNA (HVS1 & 2 *mt*DNA) for determination of maternal affinities, diversity in the hypervariable region and availability of haplogroups, in different tribes. Each one of the dental casts was analyzed for a set of 17 tooth-trait combinations scored 150 individuals (75 males and 75 females) of each tribe in accordance with the Arizona State University Dental Morphology System. Only scorable samples from 393 individuals belonging to five ethnic groups i.e. Awans, Gujars, Karlals, Syeds and Tanolis were analyzed and included in this dissertation for elaborating the internationally available 22 populations of 2,433 prehistoric and living individuals of the region through Hierarchical Cluster Analysis, Neighbor Joining Cluster Analysis, Multidimensional Scaling and Principal Coordinates Analysis. Results of the dental trait analyses revealed highly consistent patterns across the data reduction schemes. All the ethnic groups of Abbottabad and Mansehra clustered separately and shared no affinity with the prehistoric Central

Asians, the prehistoric inhabitants of the Indus Valley or living communities of peninsular India. Though all the tribes has retained their own identity and seems to be polyphyletic in origin; the Syeds, Gujars and Karlals proved more closely related to one another among the analyzed tribes. Results of the hyper variable sequences of mitochondrial DNA (*mt*DNA) of individuals from all the seven tribes analyzed for determination of maternal affinities through diversity in the hypervariable region and haplogroups diversity with respect to hypervariable sequence I (HVSI) analyzed from 223 individuals of the 7 tribes revealed 83 haplotypes with the 39 unique one. The haplogroup H proved the most frequent containing 40% of the analyzed people followed by haplogroup M which was recorded in 21.8% of the sampled populations. Results of the hypervariable sequence II (HVSII) obtained from 298 individuals of the seven tribes yielded 78 haplotypes. The most frequent haplogroup with respect to HVS II was haplogroup R, which was represented by 53 percent of the sampled population. The R group was followed by haplogroup M with 32% of individuals. The mega haplogroups recorded for the major ethnic groups of Abbottabad and Mansehra were H, R and M. These haplogroup distributions among the analyzed samples revealed the genetic lineage of people of Pakistani ancestry, with components from West Eurasia, South Asia, and a very limited contribution from Sub-Saharan haplogroups. The results provide a genetic baseline for understanding the biological affinities of the selected tribes of Abbottabad and Mansehra, and can be used as a useful source for forensic examination, molecular anthropology and population genetics of the people of the area.

Chapter-1
INTRODUCTION

1.1 Origin and Distribution of Human Race

Review of the available information shows that nearly 60,000 years ago, modern humans emerged from Africa and spread rapidly into the various parts of Europe and Asia (Stringer, 2012). The migrations of early human beings began about 1.8 million years ago and *Homo erectus* first migrated out of Africa over the Levantine corridor and Horn of Africa to Eurasia. The movement of *H. erectus* expansion out of Africa was followed by *Homo antecessor* into Europe around 800,000 years ago which was followed by *Homo heidelbergensis* around 600,000 years ago, where they possibly evolved to become the Neanderthals (Finlayson, 2005). Around 200,000 years ago the modern humans, *Homo sapiens*, evolved in Africa and about 125,000 years ago reached the Near East, from where these populations spread east to South Asia by 50,000 years ago and 40,000 years ago to Australia (Bowler and James, 2003). Some genetic evidence shows migrations along two routes out of Africa, whereas some other studies suggested that a single migration occurred, followed by rapid northern migration of a subgroup. In West Asia, the people who took the southern route, spread generation by generation around the coast of Arabia and Persia until they reached India. One of the group went north (east Asians were the second group), ventured inland and radiated to Europe, eventually displacing the Neanderthals (Maca-Meyer *et al*., 2001). They also radiated from Central Asia to India. Reaching Australia between 55,000 and 30,000 years ago the

former group headed along the southeast coast of Asia (Bowler and James, 2003) while some estimates assigning it about 46,000 to 41,000 years ago.

It has been reported that about 60,000-70,000 years ago, early human migration occurred from Africa to southern Asia and Australia along the coastal regions of the Indian Ocean, a route on which Pakistan is situated (Lahr and Foley, 1994). The Indo-Pak subcontinent spreading over an area of more than one and a half million square miles, extends from the Hindu Kush Mountains and Baluchi Hills on the west, the great Himalayas on the north, to the Burmese mountains on the east and the Indian Ocean on the south. In Indo-Pak subcontinent the earliest traces of habitation are the stone tools found scattered in the Indus Valley especially around the Soan River Valley in Pakistan. These tools are the only archeological evidence of Paleolithic humans in the North of India, sometime between 200,000 and 400,000 years ago (Wolpert, 1997). Stone implements different from the flakes of stone found in Pakistan have also been discovered in southern India and thus there is evidence for at least two distinct, separate regions of human habitation developing at that time in South Asia.

The second great wave of human migration from East Africa or Southern Europe to South Asia took place during the Mesolithic age around 30,000 BC. Microliths have been found scattered across the Deccan plateau and the Punjab while these tiny stone weapons, called pygmy tools, closely resemble those found in France, England and East Africa, appears to have been brought to South India by hunters and food

gatherers who were quite different from South Asia's Paleolithic pioneers (Wolpert, I997).

1.2 The Pakistani Civilization

Historical review based upon the archeological remains available elsewhere shows that Pakistani land has given birth to two famous ancient civilizations viz. Gandhara and Indus Valley civilizations. The earliest Neolithic settlement of South Asia i.e. Mehargarh (Jarrige, I991) shows that settlements flourished around the same time as the earliest settlements in Mesopotamia, dating back to 7000 BC. Neolithic burial sites have also been found at Rawat, near Rawalpindi (Hussain, 1997). The ruins of the ancient city of Harappa and its southern sister city Mohenjo-Daro provides historical information regarding the people of ancient Pakistan (Marshall, 1924; Wheeler, 1947). The archeological remains bear a much more technologically advanced civilization than had been previously considered. This civilization ranks third among the earliest of the great world civilizations after those of Egypt in North Africa and Sumeria in southern Iraq (West Asia) while geographically it far exceeds both of them, covering an area at least twice that of Egypt and four times that of Sumeria. The people of Indus valley built well planned towns with agriculture based economy and had also established maritime contact for trade with Mesopotamia and Sumeria for over a thousand years as shown by recently discovered Harappan outposts along the Makran coast, including that of Sutagen Dor near the border of Iran (Dales and Kenoyer, 1991).

The widespread distribution of the culture of the Indus Valley Civilization, extending over half million square miles from the borderland of Baluchistan to the deserts of Rajastan, and from the Himalayan foothills to the tip of Gujarat shows the strength of the people of the ancient Pakistan. The careful analysis of skeletal remains at Harappa shows that the people of Indus Valley were Proto-Australoid and Mediterranean type as in modern peninsular India (Wolpert, 1997). The Indus Valley Civilization collapsed around 1500 BC due to a series of earthquakes and floods around 1700 BC which devastated Harappan agricultural systems of the Indus Valley Civilization (Dales, 1986). The tribes of the original Indo-European-speaking, seminomadic barbarians from the region between the Caspian and the Black Sea, were driven from their homeland in West Asia around 2000 B.C. Driving with them their herds of cattle, sheep, cow and horses, these tribes moved in every direction and opened a new chapter in the history of Europe as well as South Asia, the traces of these great nations are available here and there as names of countries, cities etc. The first Indo-Europeans were the Hittites that settled in a new homeland as traces of them are found just south of Caucasia around 2000 BC and other tribes pushed on, some to the west, across Anatolia and some to the east toward Persia. They remained settled in Iran for some time following their long migration, by 1500 BC they split up once more and the pastoral tribes known as the Aryans, advanced still further east over the Hindu Kush Mountains and conquered the Indian sub-continent (Wolpert, 1997). The dark skinned pre-Aryan inhabitants, already in decline and were unable to defend themselves against the fairer-skinned Aryans

who possessed superior weaponry and had the use of harnessed horses. It was the most important invasion in Indo-Pak history. Since the Aryans brought with them not only their Caucasian genes but also a new language, Sanskrit, new gods and the three-caste social structure; priests, warriors and commoners. Invasions were often repeated in the history of the Indo-Pak Sub-continent and during the centuries this land has witnessed a succession of invaders like Georgians, Persians, Greeks, Turks, Arabs and Mongols. From Central Asia a series of invasions including those led by the Indo-Greeks, Indo-Scythians, Indo-Parthians and Kushans in the North-Western Indian Subcontinent from 180 BC took place (Trivedi, 2001; Bamshad, 2011). These invasions have added to the cultural and genetic diversity of the Indo-Pak Sub-continent.

1.3 The Study Area

Pakistan has a diverse polyphyletic profile of communities distributed into variety of ethnic groups, having variety of cultures, languages and geographical backgrounds, which make this land suitable for unraveling early human migrations, population study and evolution history. The 21st century's Pakistan consists of four provinces along with Islamabad capital territory and the Federally Administered Tribal Areas (Jaffery and Sadaqat, 2006). Khyber Pakhtunkhwa (KP) province, the project area, is one of the five provinces of Pakistan, located in the northwest of the country. Since ancient times numerous groups have invaded KP including the Persians, Greeks, Scythians, Kushans, Huns, Arabs, Turks, Mongols, Mughals and the British. Khyber Pukhtunkhwa lies in the region. The province was named North-West Frontier Province during the colonial

period when it formed the northwestern frontier of British India. The Province has an area of 100,140 km², and was recently renamed as Khyber Pakhtunkhwa.

The Province has seven divisions among them Hazara region is located in the northeast of the Province. Hazara comprises six districts viz. Abbottabad, Battagram, Haripur, Mansehra, Kohistan and Torghar. Population of Hazara region is heterogeneous and consists of Hindkowans, Pashtuns, Gujars and Kohistanis (Truman 1909; William, 1910; Watson, 1907). Hindkowans mostly live in Abbottabad, Haripur and Mansehra districts while Pashtuns live in Battagram and Torghar Districts, Sirikot valley of North Eastern Haripur and Oghi and Agror valleys of North-Western Mansehra and Dard Kohistanis live mostly in Kohistan District. The origin of the name Hazara is obscure and has been identified with Abisara, the country of Abisares, the chief of the Indian mountaineers at the time of Alexander's invasion (Imperial Gazetteer of India, 1931). Its name has also been derived from Urasa, or 'Urasha', an ancient Sanskrit name for this region (Kalhana and Stein, 1973; Rajatarangini, 1988). The Hazara region population was estimated to be over 4.5 million in 2005 and its total area is 18013 km².

Two major languages are spoken in Hazara region Hindko and the Pashto along with Gojri and Kohistani. Hindkowans speak Hindko as their mother tongue including Kaghani and other Northern Lahnda dialects are spoken by the majority of population in Abbottabad, Haripur and Mansehra Districts. The Pashtuns speak Pashto which is spoken by the majority of population in Battagram, Tor Ghar and in some parts of Mansehra District. Kohistani is spoken in Kohistan District whereas Gojri is spoken by

most of the Gujar tribes of Kohistan, Battagram, Torghar, Abbottabad and Haripur Districts (Awan, 2009). According to official information Hazara has an ethno-linguistic majority of Hindko and Northern Lahnda-speakers of around 60% whereas about 40% speak Pashto and other languages like Kohistani, Gojri etc. (Anonymous, 2001). Abbassi, Awans, Dhund, Jadoon, Gujar, Tanoli, Turks, Karlal, Syed, Swati, Utmanzai, Khawaja, Sulemani, Rajputs, Maddakhel, Kohistanis, Tareen, Qureshi, Mashwani, Piracha, Mir, Mughals, Tahirkheli, Kashmiri, Dilazak, Shilmani and the Isa Zai tribe of Tor Ghar District are the main tribes of Hazara region (Truman, 1909). Keeping in view the limitations of time resources and scope of the study only two Districts of Hazara were included in this endeavor. Summary of the anthropology and demography of the districts is given below:

1.3.1 The Abbottabad District

Abbottabad District occupies the central position of Hazara Division Khyber Pakhtunkhwa Province of Pakistan. The terrain of Abbottabad is rugged mountainous with scenic beauty and mild pleasing environment of most temperate nature. Abbottabad is situated between 33° 50′ and 34° 23′ North and 73° 35′ and 73° 31′ East, surrounded by Mansehra District in the North, Muzaffarabad in the East, Rawalpindi District in the South East and Haripur District in the South West (Figure 1). The district is spread over an area of 1,967 km^2 with an average elevation of mountain peaks of 2,500 m to 2,700 m, the highest being Miranjani peak of 3,313 m height from the sea level (Pastakia, 2004). Abbottabad District is a unique rich biogeographic region having

multifarious floral and faunal wealth. Forests cover 36,394.6 ha, amounting to 21.4% of the district's total area (Aziz, 1950). Subtropical Chir pine forests and moist temperate blue pine forests primarily represent the vegetation of the study area. The famous Ayubia National Park of District Abbottabad spreads over 3,312 ha and was established in 1984 with the aim of preserving nature and natural processes in a viable representative area of the Gallies forests. The climate of Abbottabad is moderate in summer season and severe in winter with heavy snowfall on high altitudes. Most of the land is rain-fed, with 60% of average precipitation received during the July–August period and the remaining 40% unevenly distributed between September and June. Livelihood of the people of Abbottabad economy depends heavily on natural resources, tourism and subsistence agriculture.

Abbottabad city, the central location of the District Abbottabad is located some 100 kilometres away from Islamabad and 217 kilometres from Peshawar. The district is named after Major James Abbot, the first Deputy Commissioner of Hazara (1849-1853). The population of District Abbottabad was 1.05 million in 2008 (District Profile: Northern Khyber Pakhtunkhwa). The tribal affiliations heavily influenced the social structure of Abbottabad. Awans, Dhunds (Abbassi), Karlals, Gujars, Sarara and Kashmiris are the major ethnic groups of Abbottabad District (District Census Report of Mansehra, 1998).

1.3.2 The Mansehra District

The Mansehra District is located in North Eastern part of Abbottabad (Figure 1). Mansehra District has an area of 4,579 km^2 and the total population of 1,152,839 individuals (District Census Report of Mansehra, 1998). Mansehra is known as Takht-e-Hazara (Throne of Hazara Division) for its beauty, richness and glory (Ali, 2006). It has Kohistan and Batagram Districts in the north, Muzaffarabad District of Azad Jammu & Kashmir on the east (Figure 1). In the south are Abbottabad and Haripur Districts and in the northwest, Torghar Districts. District Mansehra lies from 34°-14´ to 35°- 11´north latitudes and 072° -49´ to 074° 08´ east. The District Mansehra topography is dominated by the high mountains, varying in altitude from 2000 meters in the south to over 4500 meters above the sea level. Mountains of great height inhabit the northern part of the district exhibiting the great Himalaya range along the boundary of Kohistan District (Ali, 2006).

Many tribes inhabit the District Mansehra, broadly divisible into the ancient peoples like Gujars and Kohistanis and the recent migrants like Pathans of the area. Awans, Gujars, Swatis, Syeds and Tanolis are the most prominent ethnic groups of the district. In addition the Akhun Khels, Dhunds, Hassanzais, Mughals, Nusrat Khels, Qureshis, Rajputs, Turks and Utmanzais are commonly mentioned ethnic groups of Mansehra (Ghulam, 2003). Hindko, Pashto and Gojri are the local languages of Mansehra while Urdu being the national language is spoken and understood throughout the district. The current growth rate is 2.4 per cent. Density per square kilometre is 252 persons

(District Census Report of Mansehra, 1998). It is reported that 45.5% of the people are under 15 years of age, 50.6% are between 15 and 64 years, while 3.9% comprise persons of 65 years old and above (District Census Report of Mansehra, 1998). The literacy rate of District Mansehra is 36.3%. It is 50.9% for male and 22.7% for females. The literacy rate is much higher in urban areas as compared to rural areas both for male and female i.e. 44.3% and 19.9%, respectively.

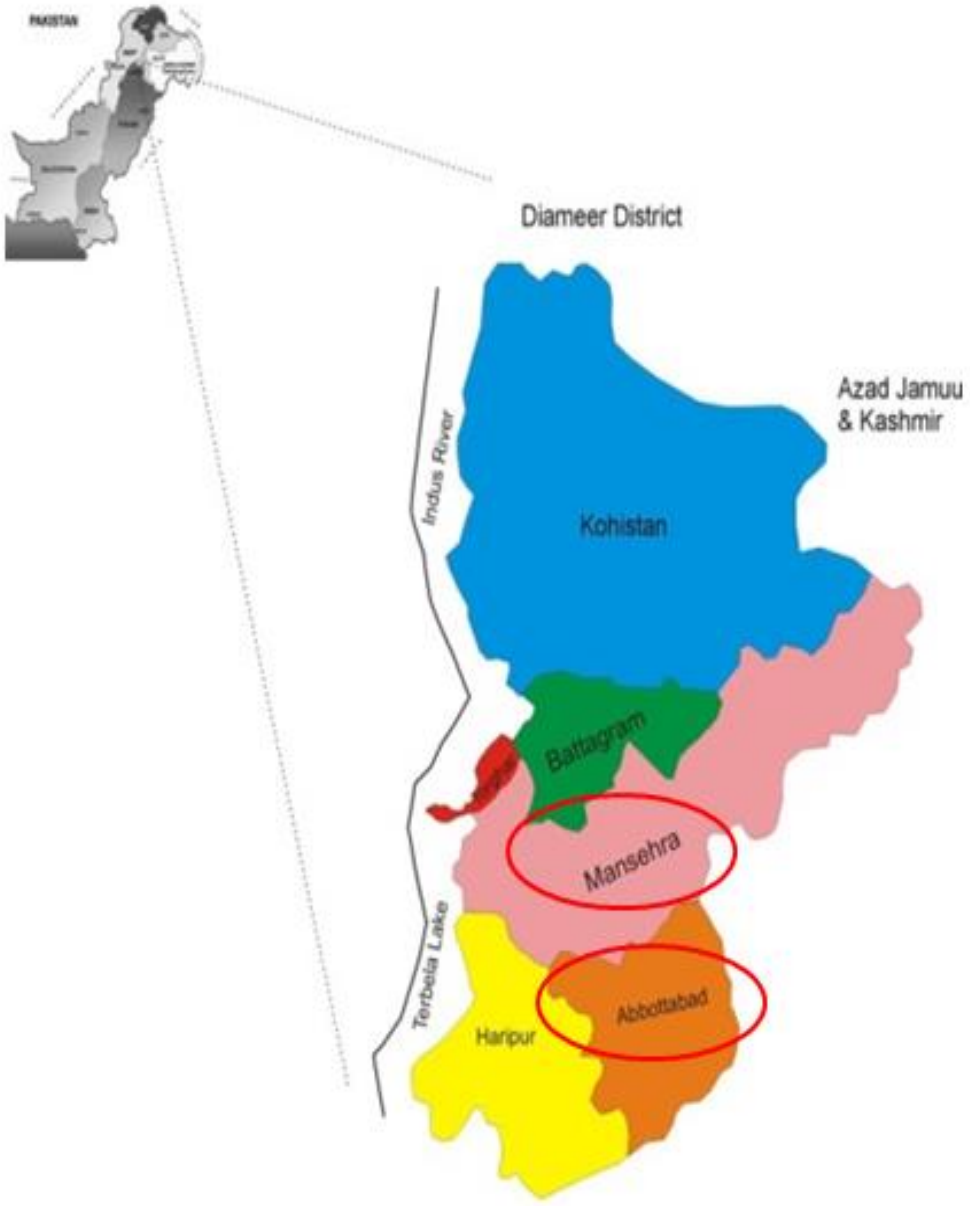


Fig.1. Location of Mansehra and Abbottabad with respect to Northern Pakistan

1.4 The Focused Groups

A brief historical review of the ethnic groups taken into consideration for this dissertation is provided below:

1.4.1 Abbassis

According to the available information the Abbassi people form a major ethnic group found throughout Bakote circle of Abbottabad, Poonch District, Bagh and the Muree Hills area. According to local perception, the Abbassis have Arabic origin and descent from paternal uncle of Prophet (PBUH) the Abbas Ibn Abd al- Muttalib. Their ancestor Takth Khan came with Taimúr to Delhi where he settled. His descendant Zorab Khan went to Kahuta in the time of Shah Jahan and begat the ancestors of the Dhond, Sarrara and Tanoli tribes of Hazara Division. The Dhond tribe forms a large part of the population in Bakote circle and Murree hills. By population, Abbassis form one of the major ethnic groups of Abbottabad District. They speak Hindko and are all Sunni Muslims. Their dialect is sometimes called 'Dhondi and Kareali (Ghulam, 2003).

1.4.2 Awans

According to the local description, Awans are a South Asian tribe of Arabic origin (Shaw, 2000) living in different parts of Pakistan. According to Awan belief, they are the descendants of the fourth Caliph Ali and a number also adopt the title, Alvi (Haider, 1896). It is believed that Awans are the descended of Qutb Shah from Herat-Afghanistan, who served in the army of Mahmud of Ghazni. He is described as Hashemite descendant of the Prophet Muhammad's cousin and son-in-law, Hz. Ali (but by a wife other than the Prophet's daughter, Fatimah (A Punjab District Gazetteers:

Attock District, 1930, 1932) but Sir Lepel Henry Griffin states that all branches of the tribe are unanimous in stating that they originally came from the neighborhood of Ghazni to India and all trace their genealogy linked to Hazrat Ali the son-in-law of the Prophet. Qutab Shah, who came from Ghazni with Sultan Mahmud, was the common ancestor of the Awans (Griffin, 1865). The Imperial Gazetteer of India however reports that they are essentially a tribe of the Salt Range; they once held independent possessions of very considerable extent and in the western and central portions of which they are still the dominant race. Ibbetson (2001) states in his book, 'Punjab Castes' that the Awans of Jalandhar claimed that their ancestors served in the armies of the Slave Dynasty and the Khilji Dynasty during the Delhi Sultanate period, which brought them from the Salt-Range. According to Denzil Ibbetson, Awans may well have accompanied the forces of Babur and the Awans of Jalandhar, who claimed to have shifted from the Salt Range at the behest of one of the early Emperors of Delhi, were particularly notable for being in the imperial service at Delhi. On a rural level, many Awan families are good farmers and cultivate land. They often carry titles typical to Punjabis who own tracts of ancestral land such as Malik, Chaudhry and Khan (Rose, 1997). The modern surname system often results in members of the same family with different surnames, some choosing their position as a surname i.e. Malik or Chaudhry, and some choosing their clan, tribe and family name of *Awan*. Though the origin of the Awans may be a matter of some debate, it has long been recognized that the composition of the tribe is wholly Muslim. The most extensive study of the tribe was recorded during the British Raj era, and as a result of census data assembled during this

period; the Awan tribe was invariably classified as being exclusively Muslim. The Awan population is found nearly everywhere in Khyber Pakhtunkhwa especially in Hindko speaking areas of Hazara Division. Awan resides in a small number in Azad Kashmir and a lesser extent is also present in Sindh and Baluchistan. Awans can also be found in Afghanistan and some parts of India (Douie, 2003). A comprehensive and more realistic description of Awans is available in the *A Glossary of the Tribes and Castes of thePunjab and North West Frontier Province* (Weston, 1911).

1.4.3 The Gujars

Gujar is a wide spread ethnic group of people in India, Pakistan and Afghanistan (Lalata *et al*., 1971). Varioous theories about the origin of Gujars are available. Some people think that Gujars appeared in the Indus Valley (Pakistan) and Northern India at the time of the Huna invasions or Gujar also called Aryas, arrived here some to 242- 300 BC. Wikely (1985) suggested that Gujars invaded India in third century B.C. but according to Nidvi (1985) they came to India from Gujarustan (Gujarustan or Gorgia) in 5th century. It is believed that the Gujars originated in the Indus Valley and from here they spread out all over the world but according to Rana Ali Chohaan (1960) they were living before ten thousand years ago in the Indo Pak Subcontinent. For the first time the word Gujar was used by Ramchand (Chohann, 1960). The spread of Gujars in the subcontinent can be realizeddfrom the the facts that "*the Gujars are often said to have 84 clans or sections and in Ludhiana their Mirasis address them as ' Chaurasi got da diwa, ' i. e. 'Light of the 84 clans' ; but other accounts assign them 101, 170 or even 388 sections (Weston,*

1911). According to Chohaan (1960) Gujars are aborigines to Indo-Pak, which he confirmed from Mahbarat. Puri (1971) reported that Gujars entered into circle of Islam during Mughal period and after accepting Islam the area of Balakot (named after Bala GujaR), still remained under their control but in seventeenth century howerer Balakot was occupied by Swatis (Puri, 1971). In Pakistan the Muslim Gujars are considered as a tribe while the Hindu Gujars are assimilated into several Varnas of Hinduism (Parishad and Bharatiya, 1996). According to other reports, Gujars are primarily an amalgamation of local South Asian people who have emerged from Central Asian elements over time. Many Gujars are well represented in agriculture, civil service and the urban professions and are established land lords on large tracts of lands in northern India and Pakistan. In Pakistan about 33 million Gujar people are reported while in India their population is more than 30 millions. Gujar populations are spread all over the country in urban and rural areas of of all the five provinces. They are found everywhere in Hazara division especially in the Mansehra, Haripur and Abbottabad districts. They once owned a tract of 84 villages in the center of Hazara including the Channai Hazara. The chief of Gujar Tribe of Hazara was the ever-mentioned Mokaddam Mir Ahmad Gujar, the jagirdar of Kot Najibulla (Watson, 1907). Gujars are in simple majority in Mansehra and Haripur Districts, especially in Kaghan Valley (Ali, 2006).

1.4.4 The Jadoons

The Jadoon ethnic group also called Gadoon is the Pashto speaking population. Horace Rose (1911) a British ethnologist reported Jadoons being present partly in Gadoon area

of district Swabi and partly in Abbottabad and Haripur Districts, while some members of the tribe live in Nangarhar and Kunar Afghanistan. The Jadoons speak Pashto in Swabi and Afghanistan while speak Hindko in Abbottabad and Haripur and are divided into two clans i.e. Salar and Mansoor (Rose, 1911). The Salar clan includes Mastkhwa Zai, Atho Zai, Isa Khel, Abdul Rahim Zai, Ali Sher Khel, Qalandar Khel, Dowlat Khel, Awd Khel, Ali Khel, Muhammad Khel and Suleman Zai sub tribes. The Mansoor clan include sub tribes viz. Mthi Zai, Aldar Zai, Wali Khel, Qaasam Khel, Shammi Khel, Allah Dad Khel, Khawaja Rustam Kor, Adin Zai, Omar Zai, Gawar Zai, Isa Khel, Dolat Zai, Yaqub Zai, Shaan Zai, Hassan Zai, Bahol Zai, Khor Zai, Shayeb Zai, Mama Zai, Khalil Zai, Amra Zai, Hassa Zai.

Originally the Jadoons lived on the western slopes of the Spin Ghar ranges and in the Nangarhar region of Afghanistan. Later on, they migrated to the Kabul region of Afghanistan. Jadoons joined the Yusufzai in 16th century, who had been expelled from Kabul by Mirza Ulugh Beg who was the paternal uncle of the Mughal emperor Babur, and they migrated eastwards to the Peshawar and settled in areas that inhabited by the Dilazak tribe of the Afghans. They defeated the Dilazaks at the Battle of Katlang and pushed them east of the Indus River to the Hazara region. Eventually they settled in Swabi District at the western bank of the Indus River. Later on some of the Jadoons also settled on the eastern bank of the Indus River, in Abbottabad and Haripur Districts. Weston (1911) justifies the word Jadun as a derivitive of the Jadu or Yadu Rdjputs. The details regarding their social and anthropological profile can be read in Watson (1907).

1.4.5 The Karlals

The Karlarr ethnic group has a restricted population found only in Abbottabad and Haripur Districts of Khyber Pakhtunkhwa province. Locally the people trace their descent from Karlal Shah. The majority of the Karlals is Sunni Muslims and is extensively distributed in Galiat area of Abbottabad District. The Karlarr tribe speaks the Hindko language and consists of the Nara ilaqa in Abbottabad. It is reported that they were formerly the subjects of the Gakkhars, from whom they released themselves about two centuries ago. Other people believe that they are the descendants of Sardar Ranjeet Singh. Whatsoever the reality is, they are very peaceful people having a lot of gender based division of labor and industries indeed. They are associated with Jadoon, Abbassi and Gujar tribes in Abbottabad and enjoy a respectable position in the society.

1.4.6 The Syeds

Syeds are the descendants of Prophet Muhammad (PBUH). It is a general consensus of the Islamic history researchers and researchers of the Prophet's genealogy (PBUH) that Prophet Mohammad's family (PBUH) has its roots in the family of Prophet Ismail (PBUH), the son of Prophet Ibrahim (PBUH). Mohammad (PBUH) is an adherent of the Prophet Ibrahim's religion (PBUH), a descendant of Ismail (PBUH) from an Arab noble tribe of Quraysh, Hashimite clan. The Arabic word "Al-Sayyid" (colloquially Syed) coincide to the English words "lord, chief, or leader". Sayyid is the title given in Islamic culture to the descended people from the grandsons of the Prophet Mohammad (PBUH) through his daughter Hazrat Fatima. Hazrat Fatima was the only one to live after the Prophet (PBUH) died. The Sayyid's ancestors migrated to Indo-Pak from

different parts of the Arab world, Central Asia, Iran and Turkestan, during the incursion of Mongols and other periods of turmoil, during Mahmud Ghaznavi period, Delhi Sultanate and Mughals and until the late 19th century. Some early migrant Sayyids moved to the Deccan plateau in the time of the Bahmani Sultanate and later Qutb Shahi kings of Golconda, Nizam Shahi of Ahmadnagar and kingdoms of Bijapr, Bidar and Berr. Some visited India as merchants and some migrated from Abbasid, Umayyad and Ottoman Empire. They ruled over India during the Delhi Sultanate during the short-lived period of 1414-1451. Their name figures prominently in Indian history at the fall apart of the Mughal Empire, when the Sayyid Brothers created and dethroned Emperors at their will (1714–1720). The new British colonial authorities that replaced the Mughals after the Battle of Buxar in 1764 worked with various Sayyid jagirdars. More than 15 million South Asians claim descent from the Prophet's (PBUH) tribe, approximately 3 per cent of the Muslim population of South Asia. The Indo–Pakistan–Bangladesh area of Asia have the most number of Sayyids, namely seven million in India, less than seven million in Pakistan , one million in Bangladesh and seventy thousand in Nepal. The Syeds came to Mansehra along with Jalal Baba and Syed Ahmad Shaeed's Mujahidin. Syed Jalal Shah was the son-in-law of the last ruler of Turkish dynasty, Sultan Mehmud Khurd. Syed Jalal Baba, a descendent lived in Kaghan and Swabi Maira. Syed Jalal Shah (Jalal Baba) conspired with Swatis and invited them to invade (Pakhli Sarkar). They succeeded to overthrow the Turkish rulers from the Sarkar. The most crucial attack was that of the Swatis under the command of Jalal Baba in 1703 A.D. They ousted the Turks and captured this area (Suneela and Frayal, 2011).

During the Sikh regime, Punjab was under the control of Maha Raja Ranjit Singh, who had also extended his authority into the North West Frontier. In 1826 Syed Ahmad Shaheed launched jihad against the Sikh rulers, his headquarter was near Peshawar but was martyred in 1831 by the Sikhs along with hundreds of his troops and followers in Balakot and Mansehra (Ahmad, 1987). The Syeds are settled everywhere in Mansehra and are respected due to their noble descent. The Syeds belong to Bukhari, Gilani, Mashhadi and Tirmazi sections etc. (Suneela and Frayal, 2011).

1.4.7 The Tanolis

Tanoli occupy mostly the Tanawal area of Haripur, Abbottabad and Mansehra while some families are also found in Haripur and Abbottabad Districts. They are present in Ghazni and Paktia Provinces of Afghanistan also. There is a controversy about the fact that whether the Tanolis are Afghan Pashtuns or Barlas Turks, because they are sometimes recognized as a Barlas Turkic tribe related to the Mongols, who acquires the Pashtuns lifestyle to an extent and have assimilated many Pashtun cultural features. The Tanolis participated in the frontier wars during 1840s against the British in alliance with other Pashtuns of the region at the time of British Raj. The Tanolis were described as “extremely hostile”, “brave” and accounted for the best swordsmen in an analysis of Charles Allen's about these wars (Watson, 1907).

It is believed that Mr. Amir Khan Beerdewa, the founder of the Tanoli tribe, had six sons, namely Pall Khan, Hind Khan, Thakar Khan, Arjin Khan and Kul Khan and the six clans of the Tanoli are named after the names of Mr. Beerdewa's sons. It is said that

the Tanolis originated Dara Tanal, in the Ghazni region of Afghanistan and joined the army of the Ghaznavi emperor Sabuktigin and traveled with them to India in the 10th Century. After the invasions, the Tanolis settled in Swat and Buner where they formed their own state and appointed Anwar Khan Tanoli as their first head but later they came into conflict with the other Afghan tribes who had newly migrated eastward into the region, most notably the Yusufzai. The Tanolis were defeated at a battle in Topi under their leader Ameer Khan and were forced to cross the Indus River in search of a new dwelling place. Under the command of Maulvi Mohammad Ibrahim, they crossed the River Indus and settled on the eastern bank, capturing the territory after defeating the Turk lashkars. Their settlement took place in 1472 under the leadership of their prominent leaders "Charra and Mamarra". The two brothers divided the area into two parts, the upper and the lower Tanawal. Two main Tanoli clans, the Hindwal and the Pallal, fell into a dispute and had a nasty tussle between them in the 18th and early 19th century. The Hindwal clan gradually gained superiority, Mir Painda Khan of the Hindwal clan successfully united all Tanolis into one entity and that eventually became the princely states of Amb and Phulera. The Amb State lasted until 1969, with its primary capital at Darband and summer capital at Shergarh. Haibat Khan and Suba Khan, after eleven generations, became prominent Khans of whom former founded Amb state and his grandson, Painda Khan, became independent master of the Hazara region (Watson, 1907). The Tanolis living in Pashtoon-dominated areas of Khyber Pakhtunkhwa and Afghanistan speak Pashto language, while their mother tongue is Hindko in Hazara Division of Khyber Pakhtunkhwa.

1.5 Ethnicity

A tribe can be defined as a small group of people who share a culture, speak a common dialect and share a perception of their common history and exclusivity (Hunter and Whitten, 1976) or a tribe is a regional population in which, there is kin or non kin groups with representatives in a number of native groups (Ember and Ember, 1999). Whereas ethnicity is a multifactorial concept which is embraced of cultural constructs, ecological specialization, genetic background and self-identification (Crews and Bindon, 1991). Ethnicity is a group of individuals identified on the bases of race, colour, language and region according to Cambridge Encyclopedia (Cashmore, 1998). The Encyclopedia Britannica defines it as a social group or kind of population that in a larger society is set apart and destined together by common links of race, language, nationality or culture. Generally at the core of ethnic identification is what has been termed as prehistoric affinities and attachments derived from the place of birth, kinship relationship, religion, language and social constructs that come naturally to an individual that attach him to other individuals from the same background. These attachments withstand in the unconscious and can be renewed by suitable stimuli (Feroz, 1984). One can coin some common features of an ethnic group like collective name, common myth of descent, shared history and characteristics, shared culture, relationship with a specific territory, logic of solidarity, shared linguistic and mutual religion.

1.6 Profiling Human Races

The recent developments in molecular genetics has an evident influence on supplementing the available archeological, anthropological and biological information regarding modern man (Renfrew, 2000; Cann *et al*., 1987; Excoffier and Langaney, 1989; Ingman *et al*., 2000; Macaulay *et al*., 2005) and elaborate genetic differences between individual, races and ethnic groups. A number of studies clearly demonstrated that there are genetic substructures in the human populations resulting as a consequence of genetic drift and migration of sub-groups of humans and that the individuals of a certain group are genetically more similar to each other than to individuals of another group (Cavalli-Sforza *et al.*, 1994; Jakobsson *et al.*, 2008; Rosenberg *et al.*, 2002; Tishkoff *et al*., 2009). Genetic divergence has occurred due to non-random mating among isolated populations and genomic diversity within and among populations is determined primarily by mutation and neutral demographic factors like effective population size and degrees of migration among populations (Slatkin, 1987; Wright, 1951). Population subdivisions, population extension dynamics and human migration patterns are evaluated by using different molecular techniques, there is a need to value our diversity as human beings (Risch *et al.,* 2002). Several other fields have been and are still, actively studying human history and evolution in addition to molecular evolution and genetic approaches to the origin and distribution of species. The human story in the form of recorded text goes back only as far as 4,000 years. Some other methods of investigation are required to study older history. Historical linguistics and the languages which are spoken today hold the evidence of their origin and are related to the ancient languages

in an assessable manner. As the language has a relative shallow time-history and linguists have suggested that languages do not hold evidence of their origin for more than 10,000 years (Jobling *et al.*, 2004). Archaeological research provides the ability to study human history, sometimes at great time depth, through the analysis of physical remains like bones, stone tools, pottery, waste deposits, coins, inscriptions and dwellings left over by early human groups. Paleontology however, provides a very deep ancestral record of human beings. Molecular genetics research is a recent method utilized to study human history (Cavalli-Sforza *et al.*, 1994; Jobling *et al.*, 2004).

Genetic variation at the individual level helps the subject to see his past and shape his future regarding the possible implications in the field of medicine, prevention, methods, disease susceptibility and response to drug treatment etc. Several studies have revealed that the human population is not homogenous in terms of disease risk and treatment responses (Jorde *et al.*, 2001; Bamshad *et al.*, 2004). The variation among different races at genetic level is obligatory for the effective planning of prevention and treatment strategies.

In the beginning of the 20th century, genetic differentiation was explored through the analysis of ABO blood group patterning between human populations (Landsteiner, 1901). The magnitude of this genetic variation only became apparent when individual differences in proteins could be systematically studied in the 1950s to 1960s (Cavalli-Sforza *et al.*, 1994). Genetic variation could be studied directly and the field of evolutionary genetics expanded rapidly when analysis methods for the hereditary

material, DNA itself, became available (Cavalli-Sforza *et al*., 1994; Jobling *et al*., 2004). Until recently, most studies investigated the origin and dispersal of modern humans concentrated on two haploid compartments of the human genome, namely, the mitochondrial DNA and the Y-chromosome (Jobling and Tyler-Smith, 2000; Jobling and Tyler-Smith, 2003; Forster, 2004; Torroni *et al*., 2006). Some studies investigate autosomal variations. However, these studies were usually on particular genes and were under the investigation due to their influence on a specific phenotypic property or disease risk. Therefore the variation would have been subject to selection pressures. Advances in the Human Genome Project have recently allowed us to access large amount of information on neutral genetic variation that would give the more complete picture of human evolutionary history (Przeworski *et al*., 2000; Garrigan and Hammer, 2006).

1.7 Dental Anthropology

Phenetic variations in root and enamel structure in dental traits have long been recognized for their importance as phenotypic expressions of genetic differences between human groups (Carabelli, 1842; Owen, 1845; Tomes, 1876). Physical and dental anthropologists have continued to discover, describe and categorize new dental trait variation forms (Hrdlicka, 1920; Gregory and Hellman, 1926; Weidenreich, 1937; Dahlberg, 1950; Morris, 1975; Scott, 1977; Morris *et al*., 1978; Harris and Bailit, 1980; Burnett, 1998; Correia and Pina, 2002; Edgar and Sciulli, 2004; Weets, 2009). Teeth have long been appreciated by dental anthropologists to elaborate pathological conditions of ancient populations, their general health conditions, diet and even social status of

individuals (Cucina and Tiesler, 2003; Eshed *et al*, 2006; Hillson, 1979). Correspondingly dental eruption status can be used for determination of age of death of youngsters while macroscopic tooth wear can be used for determination of death age of adults (Brothwell, 1981; Smith, 1991). Microscopic tooth wear provides information about what an individual was eating close to the time of its death (Teaford and Lytle, 1996). Tooth morphology can be used in forensic sciences to identify individuals and are used for getting information about human evolution in paleoanthropology. Tooth wear, size and morphology provide important information about the diets of early humankind (Ungar and Grine, 1991) and have been used to determine hominin phylogeny (Strait and Grine, 2004). Dental morphological variation has been used to investigate relationships between modern human populations.

Dental characters prevail in the identification of most species and genera of both fossil and archeological traits. Among organs, teeth are unique in enabling direct comparisons to be made between fresh specimens formed a few months before and fossils excavated from sediments formed millions of years ago. Teeth portray their genetically inherited patterns and their evolutionary history more accurately than all other organs. This precision of genetic expression is because of their highly protected developmental environment, entrenched as they are in their submerged dental follicles up to their full morphological maturity before emerging into the potentially damaging environment. Teeth are the ultimate and amongst the most perfect extrinsic expressers of the intrinsic units of evolutionary change, the mutations of genes, by means of casting their

primeval and delicate genotypic templates into the enduringly fossilized form of highly mineralized phenotypic morphology. Underlying phenotypic dental characteristics that are directly observable, the genetics has enabled rates and degrees of gene flow to be calculated and genetic drift to be estimated in divergent populations. Most studies comparing the dental metric variation of two or more groups have focused almost exclusively on individual teeth as units of study and restricting the analysis to tooth by tooth inspection (Harris and Rathbun, 1991). Studies of nonmetric variation in human deciduous teeth fail in comparison with those of the permanent dentition (Scott and Turner, 1997). In oral cavity the arrangements of teeth determine the feeding habits of every vertebrate species. Changes in tooth shape, size and number constitute one of the major driving forces in evolutionary adaptation (Headon *et al*., 2002; Kere *et al*., 1996; Monreal *et al*., 1998, 1999). Commonly it is accepted that morphological variation among recent human populations is greater than that of genetic variation (Stringer and Andrews, 1988). In spite of extensive work on the subject, the forces that cause a tooth to erupt, to move coronally into occlusion, are poorly understood (Marks and Cahill, 1984; Steedle and Proffit, 1985; Gorski and Marks, 1992; Wise *et al*., 2002).

Dental morphology is the study of nonmetric dental traits (NDT) which involves genetically modulated trait expressions that can be used for comparisons within and among populations (Scott and Turner, 1997, 1988; Rodriguez, 2003, 2005; Rodriguez and Delgado, 2000). Dental morphology studies acquired an organized turn when Dahlberg (1956) and Hanihara (1961) established the first systematic procedures and comparative

casts for the analysis of dental traits. Turner, Scott and colleagues advancing this standardized work at Arizona State University developed the current dental morphological standards and comparative dental casts which are used all over the world for elaboration of dental morphology including the index for comparison in our study (Turner *et al.,* 1991).

Maula (1993) defines the nonmetric dental traits as morphological variants of the crown and root structures. Nonmetric dental traits vary between populations, because of which they can be used to research the ancestry of human populations. Evolutionary alterations appear quicker in the more distal teeth of every tooth group (Maula, 1993). Studies on museum material and dental models of living patients in many countries built an extensive database which is now available and has yielded broad morphological groupings that can be interpreted in terms of the migrations and ancestry of human populations (Hillson, 1996). Optimistically one could try to calculate when two populations differed from each other (Alexandersen, 1988b) using dentochronology (Scott *et al.,* 1988). In 1964, Brabant revealed that tooth morphology changes very slowly from the Paleolithic up to modern times. A tendency was found towards the decreased frequency of shovel shaped incisors, in molars with four cusps in the upper jaw and molars with five cusps in the lower jaw while the tendency towards increased frequency was found in Carabellis tubercle and in congenital absence of third molars (Brabant 1971; Alexandersen, 1988a). The oldest traits are found in most parts of the continent, while other traits develop as an adaptation to the natural environment

and subsistence (Zachrisson *et al.*, 1997). Dental traits appear to be transmitted in a simple genetic fashion, are resistant to environmental factors (except wear) and can be easily examined in large numbers of skeletal specimens (Turner, 1989, 1990).

1.8 **Mitochondrial DNA (*mt*DNA)**

The genomic era of populations has emerged in the research of human *mt*DNA (Hedges, 2000; Richards and Macaulay, 2001) by the utilization of complete or nearly complete *mt*DNA sequences to infer the prehistoric dispersal of modern humans and the phylogeny of the major *mt*DNA lineages in Europe, Africa, America, Oceania (Australia and Papua New Guinea) and East Asia (Ingman *et al.*, 2000; Finnila *et al.,* 2001; Maca-Meyer *et al.,* 2001, 2003; Torroni *et al.,* 2001; Derbeneva *et al.,* 2002*b*; Herrnstadt *et al.,* 2002, 2003; Ingman and Gyllensten 2003; Kong *et al.,* 2003; Mishmar *et al.*, 2003; Reidla *et al.,* 2003). The availability of complete mitochondrial genome has broadened its utilization as a genetic marker to investigate the time of origin and migration of humans from Africa to other continents (Ingman *et al*. 2000; Macaulay *et al*. 2005). The maternal lineage of human populations can be revealed by determining the pattern of maternal inheritance of *mt*DNA. Several other features of the mitochondrial genome, like its high copy number, absence of genetic recombination and higher mutation rate make it an efficient molecular genetic tool (Maji *et al.*, 2009). Mitochondrial DNA (*mt*DNA) is located in an extra-nuclear organelle, the mitochondria (a cytoplasmic organelle involved in energy production in eukaryotic cells). The *mt*DNA is a histone-free, double-stranded circular molecule. It is a compact genome

that encodes 13 polypeptides of approximately 80 protein subunits involved in oxidative phosphorylation, in addition to two ribosomal RNAs and 22 transfer RNAs. The human mitochondrial genome contains 16569 bp of DNA and has been completely sequenced (Anderson *et al*., 1981). It primarily consists of densely organized genes encoding rRNAs, tRNAs and proteins involved in oxidative respiration, together with a non-coding region approximately 1100 bp in length containing the origin of replication (Cann *et al*., 1987).

This non coding region approximately 1,100 base pairs long is also called the control region (Anderson *et al*., 1981). The Displacement loop (D-loop) occurs in this main non-coding area of the mitochondrial DNA molecule, and this region is also called D-loop region (Pereira *et al*., 2004). The D-loop is the major control site for *mt*DNA expression because it contains the leading-strand for origin of replication and major promoters for transcription (Miyazono *et al*., 2002). Both strands of the *mt*DNA are completely transcribed from the promoters in the D-loop. In addition to the promoter sequences, there are two small regions known as the hypervariable regions I and II (HVI and HVII) (Jazin *et al*., 1998). One strand of *mt*DNA is purine rich (termed the heavy strand) and one strand is pyrimidine rich (termed the light strand). Nucleotide positions in the *mt*DNA genome are numbered according to the convention of Anderson *et al*., (1981) with minor modification (Andrews *et al*., 1999). A mitochondrion contains between 2–10 copies of *mt*DNA, and there can be as many as 1000 mitochondria per somatic cell.

Sequence analysis of mitochondrial DNA (*mt*DNA) from different individuals reveals a higher degree of variability relative to nuclear genomic DNA (Cann *et al*., 1987**).** The

majority of this sequence variation is found within the non-coding region sublocalized to two hypervariable (HV) regions (HVI and HVII), each approximately 250–350 bases in length (Horai and Hayasaka, 1990; Stoneking *et al*., 1991). HVI spans at least from position ~16024 to ~16365 and HVII from position ~73 to ~340.

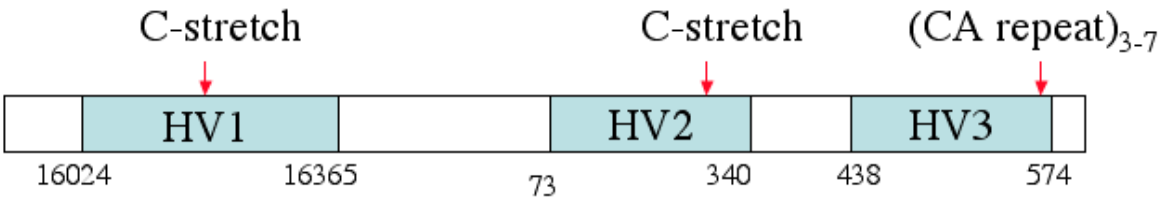


Fig.2. A sketch of the control region of mitochondrial DNA

This non-coding region of the mitochondrial genome provides an attractive target for human forensic identification studies and these regions contain the majority of mitochondrial DNA (*mt*DNA) sequence variability among different individuals (Steffen and Reena, 1998). The *mt*DNA genome especially hyper variable regions remains one of the most widely studied DNA segments in humans. It is particularly useful for studying population and evolutionary genetics, forensic and anthropological studies. Analysis of *mt*DNA is of particular importance when only degraded DNA is available, as hundreds to thousands of copies of *mt*DNA may be present per cell in contrast to the single copy of nuclear DNA (Robin and Wong, 1988). Moreover, the strictly maternal inheritance, the lack of recombination, and the high mutational rate of *mt*DNA, provide further compelling reasons for the use of mitochondrial sequences in forensic science (Wilson *et al*., 1995), population studies (Piercy *et al*., 1993), molecular evolution (Stoneking, 1994),

anthropology (Melton *et al*., 1996) and archaeology (Handt *et al*., 1994).

These regions are of interest for human identity testing because of their hyper variability consequent of their higher mutation rate and mutation is the single most important factor in producing genetic variation in *mt*DNA. Most of the sequence variation between individuals is found within two specific segments of the control region (Greenberg *et al*., 1983) the hypervariable region 1 (HVI) and hypervariable region 2 (HVII), (Vigilant *et al*., 1991; Budowle *et al*., 1999). Unlike nuclear DNA, *mt*DNA is maternally inherited (Hutchinson *et al*., 1974; Giles *et al*., 1980; Case and Wallace, 1981). Barring mutation, the *mt*DNA sequence of siblings and all maternal relatives is identical. This characteristic can be helpful in forensic cases, such as analyzing the remains of a missing person, where known maternal relatives can provide reference samples for direct comparison to the questioned *mt*DNA type (Ginther *et al*., 1992; Holland *et al*., 1993). In addition, *mt*DNA mutations are implicated in molecular evolution and human diseases (Brown *et al*., 1979). Initially, studies on human *mt*DNA were based on restriction fragment length polymorphisms (RFLPs) of either genomic DNA or *mt*DNA (Brown, 1980; Denaro *et al*., 1981; Johnson *et al*., 1983; Cann *et al*. 1987; Scozzari *et al*., 1988; Excoffier and Langaney 1989), but with the advent of polymerase chain reaction (PCR) and sequencing techniques, RFLP analysis of *mt*DNA, PCR products and sequence analysis of the highly polymorphic noncoding regions, hypervariable regions I and II, took over (Vigilant *et al*., 1991; Maddison *et al*., 1992; Templeton, 2002; Torroni *et al*., 1996, 1998; Watson *et al*., 1997; Macaulay *et al*., 1999; Quintana-Murci *et al*., 1999).

Specific features of *mt*DNA made them extremely useful also in the field of anthropology. Nowadays mitochondrial DNA is frequently used for analyzing different aspects of human population, their origin, rate of evolution and similarities and differences among individuals of same or different ethnic groups. How humans colonized the globe remains ‘‘one of the greatest untold stories in the history of humankind’’ (Goebel, 2007). Mitochondrial DNA (*mt*DNA) has been a crucial line of evidence in developing the current understanding of our genetic prehistory. Phylogenetic studies of human *mt*DNA variation support a late Pleistocene expansion of modern humans from Africa (Cann *et al*., 1987; Vigilant *et al*., 1991; Watson *et al*., 1997; Ingman *et al*., 2000). MtDNA analyses became a milestone in the complex task of unraveling earliest human migrations. It is assumed that all *mt*DNA types in the human gene pool can ultimately be traced back to a common matrilineal ancestor that lived approximately 200,000 years ago in Africa (Mishmar *et al*., 2003; Macaulay *et al*., 2005; Behar *et al*., 2008). MtDNA sequence variation thus evolved as a result of the sequential accumulation of mutations along maternally inherited lineages (Richards, 2004; Bandelt *et al*., 2005, 2006; Kivisild *et al*., 2006; Kong *et al*., 2006; Torroni *et al*., 2006; Salas *et al*., 2007). The evidence of DNA reveals that all humans are very closely related and that all surviving humans are descended from one woman who lived perhaps 200,000 years ago and called "the African Eve" or "Mitochondrial Eve". Research also shows that the story begins in Africa, home to the greatest variation in human DNA and therefore, the oldest location. Not surprisingly, people of the same ethnic and linguistic group turn

out to be genetically more closely related to each other than to the rest of the planet, but the same research shows a great deal of mixing of populations as well.

By using *mt*DNA geneticists also conduct research on racial and ethnic groups. By comparing specific DNA base pairs from members of one racial or ethnic group with those from members of another, scientists believe they can estimate how closely related the two groups are. Since changes in DNA accumulate slowly over time, the number of base pairs that differ between groups may reveal the amount of time the groups have been separated. It may also help inform scientists and historians about the historical migrations of different populations and the genealogy of families (Cann *et al*., 1987; Kivisild *et al*., 2006). Based on the mutation sites observed in the mitochondrial genome in relation to the reference sequence (Anderson *et al*., 1981; Andrews *et al*., 1999), individuals were categorized into specific monophyletic clades or haplogroups, where haplogroups represent related groups of sequences defined by shared mutations. The mtDNA haplogroups mainly fall into three macrohaplogroups, designated as L, M and N, distributions of which are geographically distinct (Quintana-Murci *et al*., 1999; Mishmar *et al*., 2003).

Several factors make *mt*DNA ideal for phylogenetic analysis over the time scale of modern humans, i.e. the absence of recombination, combined with a high copy number and fast mutation rates. A limitation, however, is that due to the inheritance from mother to child; *mt*DNA captures the history of the maternal lineage only. Another problem that arises when using only the *mt*DNA control region is that this part of the *mt*DNA genome is subject to saturation due to excessive homoplasmy because of the

rapid mutation rate. Furthermore, the distribution of mutations in the control region is non-random, leading to problematic rate heterogeneity issues when calculating divergence date estimates (Tamura and Nei, 1993; Excoffier and Yang, 1999; Meyer *et al.*, 1999). Furthermore, there is an ongoing discussion on whether human *mt*DNA evolves neutrally. There have been reports on natural selection affecting *mt*DNA, with temperature being highlighted as a possible selective force (Torroni *et al.*, 2001; Mishmar *et al.*, 2003; Ruiz-Pesini *et al.*, 2004). Several other studies, however, concluded that human *mt*DNA sequence variation has not been significantly influenced by climate (Elson *et al.*, 2004; Kivisild *et al.*, 2006; Amo and Brand, 2007; Ingman and Gyllensten, 2001; Balloux *et al.*, 2009). Despite these caveats, *mt*DNA remains by far the most widely used genetic marker in studies of human populations.

The present study was aimed at the characterization of local tribes of Abbottabad and Mansehra districts for their genetic features on the basis of dental morphological traits and mitochondrial DNA analysis. Different morphological traits have been studied from upper and lower jaws of male and female individuals of seven selected ethnic groups. The mitochondrial HVS regions of random male and female samples were PCR amplified and analyzed for nucleotide sequence.

Objectives

This study included following objectives:

1. To record haplotypic diversity for major ethnic groups of Abbottabad and Mansehra Districts.

2. To compare synoptic and haplotypic diversity for lineage elaboration of the ethnic groups.
3. To assign biological division and historic links to these ethnic groups.

Chapter-2
MATERIALS AND METHODS

Seven ethnic groups viz. Abbassi, Awans, Gujars, Jadoons, Karlal, Syeds and Tanoli residing in Mansehra and Abbottabad Districts were selected for analysis of teeth phenotypes and *mt*DNA hypervariable regions. The sampling was done in selected schools of Mansehra and Abbottabad Districts generally in isolated communities with the approval of Provincial Government, District and local school administrations.

2.1 Sampling and Analysis of Dental Impressions

2.1.1 Dental Casting

Dental casts were taken from 75 male and 75 female volunteers of each ethnic group generally in the age of 12-22 years. Appropriate informed consent for taking dental casts was obtained from participants of the concerned ethnic groups. Information about the geographical origin of their grandparents and about their first language was recorded. Students were given toothpaste and brushes and were guided to brush and wash their buccal area properly. The students were made to sit in such a way that the chances of vomiting were minimized during the casting process.

2.1.2 Selection Criteria

Research subjects were selected on the basis of four primary criteria points including age, ethnic group identity, relatedness and dental status. The selected individuals between the age of 12 to 22 years with fully erupted permanent teeth, except third molars, possessing dentitions free of dental restorations, were included for dental casting. The subjects who did not meet the four point criteria outlined above were

excluded from data collection. The subjects younger than 12 years of age or older than 22 years of age and the individuals exhibiting obvious symptoms of illness were honorably excluded from dental casting. The participants were students from secondary and higher secondary schools where the desired ethnic groups were available. Headmasters, or otherwise responsible administrative officials, of these educational institutions were approached and provided a thorough explanation of the research effort, the potential risks to subjects and also provided information about the research project. A total of 1050 school individuals met the four point selection criteria and were cast, 75 males and 75 females from each tribe (Table 1).

Table 1: Summary of the sampling

S.No	Ethnic Group	Sampling site	Gender	No. of Casts
1	Syed	Mansehra, Dhodial and Shinkiari, Mansehra	Males	75
2	Syed	Mansehra and Shinkiari, Mansehra	Females	75
3	Gujars	Attar Sheesha, Mansehra	Males	75
4	Gujars	Sandesar, Mansehra	Females	75
5	Awan	Mansehra and Dhodial, Mansehra	Males	75
6	Awan	Mansehra and Dhodial, Mansehra	Females	75
7	Tanoli	Lassan nawab, Mansehra	Males	75
8	Tanoli	Lassan nawab, Mansehra	Females	75
9	Karlal	Barmi Gali and Nathia gali, Abbottabad	Males	75
10	Karlal	Nathia gali, Abbottabad	Females	75
11	Jadoon	Havalian and Nawasher, Abbottabad	Males	75
12	Jadoon	Havalian and Nawasher, Abbottabad	Females	75
13	Abbassi	Adola and Dalola, Abbottabad	Males	75
14	Abbassi	Dalola, Abbottabad	Females	75

2.1.3 Biosafety Measures

Orthodontic disinfected dental trays were used to take dental casts with the sterilized alginate commonly used by dental surgeons. After taking the dental impression in alginate the template was filled with plaster. The alginate was used for obtaining a template due to the fact that it easily separates during removal from cast. The alginate was taken in a rubber bowl for a single impression then a little water was added to make a semi-fluid mixture and loaded immediately in the casting tray, which was of exact size of each individual subject's jaw. Then, the tray was seated into the mouth. The muscles around neck and mouth were massaged to relax the subject. After keeping the tray for 1 -2 minutes in the subject mouth it was removed from and was kept in an open area for the next step.

2.1.4 Pouring

The soft plaster was poured into the alginate templates and then the sides of the dental trays were tapped to remove any air bubbles from the plaster. Approximately 30 minutes were required for the dental plaster to set. The dry casts were removed from the impression trays and the impression material was separated from the cast carefully. Some hard plaster was left behind at the base to make it strong and stable. The casts were dried properly and were wrapped in tissue paper and preserved for further analysis. The trays were cleaned and properly treated with some disinfectant to make them ready for further use.

2.1.5 Data Collection

The dental non metric traits were identified and scored in accordance with Arizona State University Dental Anthropology System (ASUDAS) (Scott and Turner, 1997; Turner *et al.,* 1991). The ASUDAS is a series of ordinally-graded plaster plaques of world-wide variation in human non-metric dental traits. Detail regarding the ASUDAS is prepared in Appendix I. The traits mostly analyzed through ASUDAS are summarized below:

2.1.6 The Crown Traits

2.1.6.1 Crown traits of incisors and canines

The studied traits were winging of the upper central incisors (Enoki and Dahlberg, 1958; Turner, 1970), labial convexity of the upper incisors (Nichol *et al.,* 1984; Scott and Turner, 1997), shoveling as found on upper incisors, canines and lower incisors (Hardlicka, 1920; Dahlberg, 1956; Scott and Turner, 1997), double-shoveling, which occurs in upper incisors, canines and lower incisors (Dahlberg, 1956); the interruption groove appears in upper incisors (Scott and Turner, 1997), the tuberculum dentale feature is present on upper incisors and canines (Nichol and Turner, 1986), the canine mesial ridge or Bushman canine is located in upper and lower canines (Morris, 1975; Scott and Turner, 1997), the canine distal accessory ridge appears in upper and lower canines (Morris, 1975; Scott and Turner, 1997), the peg-shaped character occurs in the upper lateral incisors (Scott and Turner, 1997).

2.1.6.2 Crown traits of premolars

The traits studied include the double-shoveling located in the first premolar (Dahlberg, 1956), the premolar mesial and distal accessory cusps occurs in the upper premolars (Turner, 1967), the distosagittal ridge or "Uto-Aztecan premolar" appears in the first upper premolar (Morris *et al*., 1978).

2.1.6.3 Crown traits of molars

The traits considered were the metacone and the hypocone characters located in the upper molars (Dahlberg, 1951; Turner, 1979), cusp 5 or metaconule trait appears in upper molars (Harris, 1977), the Carabelli's trait appears in the upper molars (Dahlberg, 1956; Scott and Turner, 1997), the parastyle character is located in upper molars (Bolk, 1916; Scott and Turner, 1997), premolar lingual cusp variation is very sensitive to wear and occurs in the lower premolars (Pedersen, 1949; Kraus and Furr, 1953; Scott and Turner, 1997), the anterior fovea trait is located in the lower first molar (Hardlicka, 1924; Scott and Turner, 1997), the groove pattern feature appears in the lower molars with Y groove patterns cored for presence in this study (Hellman, 1928; Jorgensen, 1955; Scott and Turner, 1997), the cusp number was scored in the lower molars (Gregory, 1916; Scott and Turner, 1997), deflecting wrinkle appears on lower first molar (Scott and Turner 1997), the protostylid is located on the lower molars (Dahlberg, 1956; Scott and Turner, 1997), cusp 5, cusp 6 and cusp 7 are located in lower molars (Scott and Turner, 1997).

2.2 Scoring Maxillary Traits

2.2.1 Winging

A bilateral rotation of the distal margin of the incisors is termed as winging. Incisors moved or curved towards buccal side like "^"or towards lingual side like "v". It is a presence/absence dichotomy trait (Fig. 3A and B). No standard scale is available for winging and was scored 1 for present and 0 for absent.

2.2.2 Curvature

Labial convexity is described as the labial surface of the upper incisors (Turner *et al.,* 1991), when viewed from the occlusal aspect. The labial aspect of the incisors can range from being essentially flat to showing a marked degree of convexity (Fig. 3C). Curvature is the degree of arching of the labial surface of the first upper central incisors. It was studied by comparing with the standard scale.

2.2.3 Shoveling

Shoveling is defined by Hillson (1996), as in incisors, and sometimes in canines; the marginal ridges may be especially prominent and encompass a deep fossa in the lingual surface (Fig. 3D, E and F). It is the "scooping out" or enhancement of the mesial and distal ridges on the lingual surface of the incisors and rarely the canines and was studied by comparing with the standard scale.

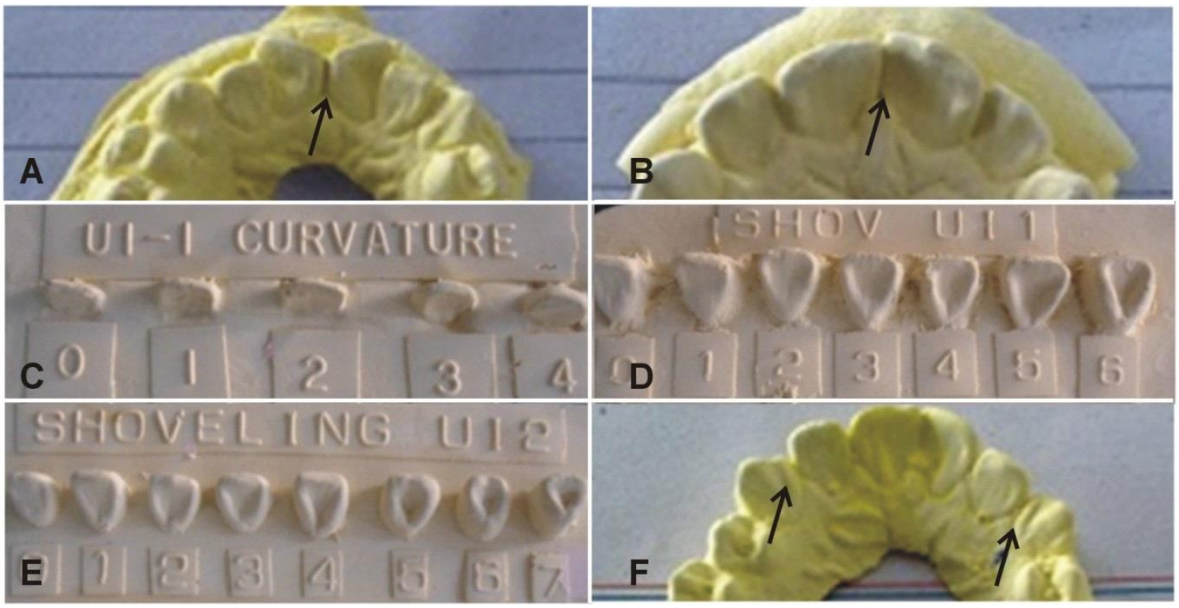


Fig.3. Scales showing **A.**Winging in central incisors move like V **B**. Winging in central incisors move like A **C.** Curvature in maxillary incisors **D.** Shovelling in maxillary central incisors **E.** Shovelling in maxillary lateral incisors **F.** Arrows pointing out the shovelling in canines

2.2.4 Double shoveling

Some incisor and canine crowns have prominent marginal ridges on their labial surfaces, a condition known as double-shoveling (Hillson, 1996), whether or not strong lingual ridges are also present. The breakpoint of the trait is grade 1 (Fig. 4A). The ridges which make the shoveling were studied and scored according to the standard scale.

2.2.5 Interruption grooves

Grooves which cross the cingulum and often continue down the root (Turner *et al*., 1991) are occasionally seen on the upper incisors (more frequently on the lateral incisor than on the central). The morphogenesis of the grooves is not understood, but they seem to be related to the tuberculum dentale. It groove may also be present at the sides

of a tooth, it does not always reach to the gums. Sometimes it is like a cut at the side ridge of tooth. It is made by the ridges of shoveling or may not be shoveling but a dent (Fig. 4B). No standard scale is available. If the groove was absent then scored 0, if present at mesial side then 1, at distal 2, both sides 3 and in mid scored 4.

2.2.6 Tuberculum dentale

This feature is situated in the cingular region of the lingual surface of the upper incisors and canines (Turner *et al*., 1991). This feature can take the form of ridges on the lingual surface or various degrees of expression of a cusp. Ridge strength, not number of ridges, is measured in grades 1-4 (Fig. 4C). It was studied using the ASUDAS tuberculum dentale plaque.

2.2.7 Canine distal accessory ridges

This trait happens on the distolingual marginal ridge of the tooth (Turner *et al*., 1991). It is one of the most difficult to score (Scott and Turner, 1997). Also this trait shows a pronounced sexual dimorphism with men having significantly higher frequencies and more pronounced expressions of the trait than women (Scott and Turner, 1997). Extra ridges are found on the lingual surface of the canines between the median ridge and distal marginal ridge (Fig. 4D). An extra ridge at the distal side was studied and scored according to the standard scale.

2.2.8 Premolar mesial and distal cusps and ridges

These are the extra cusps and ridges that span the buccal cusp of the premolar. The cusps and ridges can occur mesially, distally or on both aspects of the tooth.

2.2.8.1 Premolar Accessory cusp

Small accessory cusps are sometimes seen at the mesial and/or distal ends of the sagittal grooves of the upper premolars. These cusps are well-defined by a strong separation from both the buccal or lingual cusps (Turner *et al.,* 1991). This trait is among the most difficult to score (Scott and Turner, 1997). No standard scale is available**.** It was scored as 0 if absent, if present at mesial side then scored 1, distal 2 and on both sides scored 3 (Fig. 4E).

2.2.8.2 Premolar Accessory Ridges

Extra ridges are found on the lingual side of the buccal cusps of P3 and P4. It could be distal or mesial or both. It could be distal or mesial or both (Fig. 4F). It was scored 0 if absent, if present at mesial side then scored 1, distal 2 and on both sides scored 3.

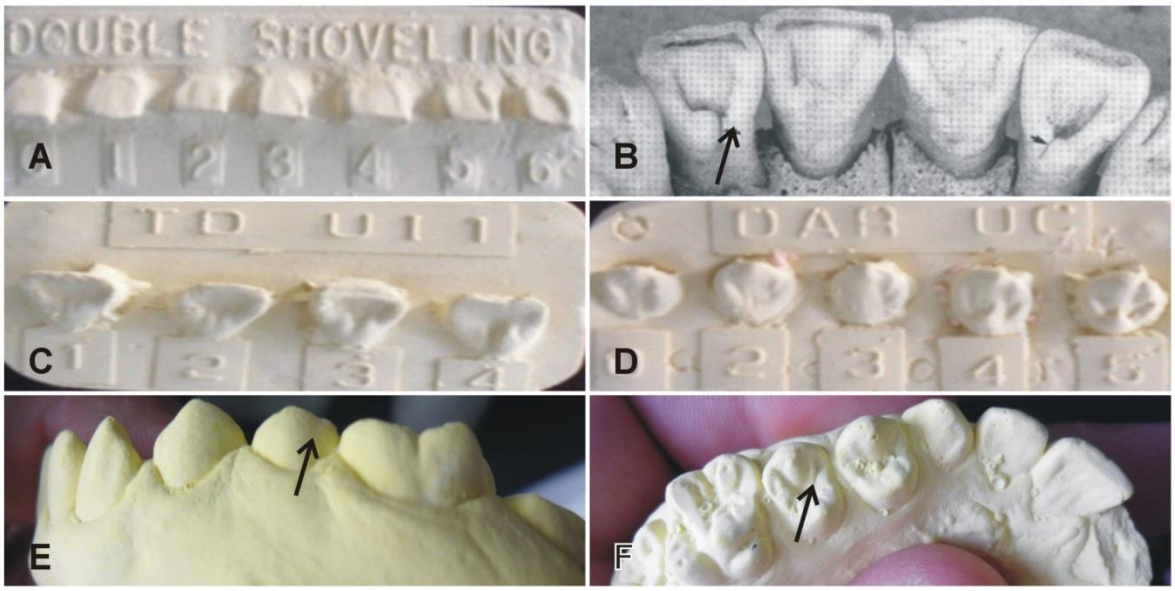


Fig.4. Scales showing **A.** Double shovelling in upper central incisors **B**. Arrow pointing out the interruption grooves in upper lateral incisors **C**. Tuberculam dentale in upper lateral incisors and canines **D.** Canine distal accessory ridges in upper canines **E**. Arrow pointing premolar accessory cusp in upper premolars **F.** Arrow pointing out premolar accessory ridges in upper premolars

2.2.9 Metacone

Located on disto-buccal aspect of the tooth, the metacone is the third cusp on the molars. Hillson (1996) explains metacone (distobuccal cusp) variations as: The distobuccal cusp is normally prominent in upper molars but occasionally it may be reduced, or absent, particularly in third molars. According to Scott and Turner (1997), scoring is based on how much the cusp is reduced. The size of third cusp (cusp 3) was studied and scored according to the scale on M1, M2 and M3 (Fig. 5A).

2.2.10 UTO-Aztecan premolar

A distosagittal ridge arises when a pronounced ridge from the apex of the buccal cusp extends to the distal occlusal border at or near the sagittal sulcus (Turner *et al*., 1991). A "triangle shape" on the tip of the buccal cusp of P3 was checked and scored according to the scale (Fig. 5B).

2.2.11 Hypocone

The hypocone (distolingual cusp) is noticeably more variable than the metacone, and is best developed on upper first molars, but reduced on second and particularly third molars (Hillson, 1996). The disto-lingual cusp was studied on M1, M2 and M3 and scored according to the standard scale (Fig. 5C).

2.2.12. Metaconule

The metaconule (a fifth cusp), may occasionally be present in the distal fovea of the upper molars between the metacone and the hypocone (Turner *et al.,* 1991). Cusp five is the occlusal tubercle on the distal marginal ridge of the molar between metacone and

hypocone(cusp 3 & 4). Cusp 5 was studied between cusps 3 and 4 on M1, M2, M3 and scored according to the scale (Fig. 5D).

2.2.13. Protoconule

The cusp between cusps 1 and 2 on upper molars is called the protoconule (cusp 6). The size of cusp 6 was studied and scored according to the standard scale (Fig. 5E). UM Cusp 5 standard scale was used by rotating it to study the protoconule on M1, M2 and M3.

2.2.14. Parastyle

The parastyle occurs on the buccal surfaces of cusps 2 and 3 (Turner *et al.,* 1991). The feature ranges from a pit near the buccal groove up to a large, well-separated cusp. It is found on all permanent upper molars, but is most common on the third and is rare on the first (Hillson, 1996). The parastyle may provide insights into dental evolution and development (Scott and Turner, 1997). It is found on the buccal surface of the paracone. An extra cusp or outgrowth was studied on the buccal surface of paracone (Cusp 1) and compared with the standard scale (Fig. 5F).

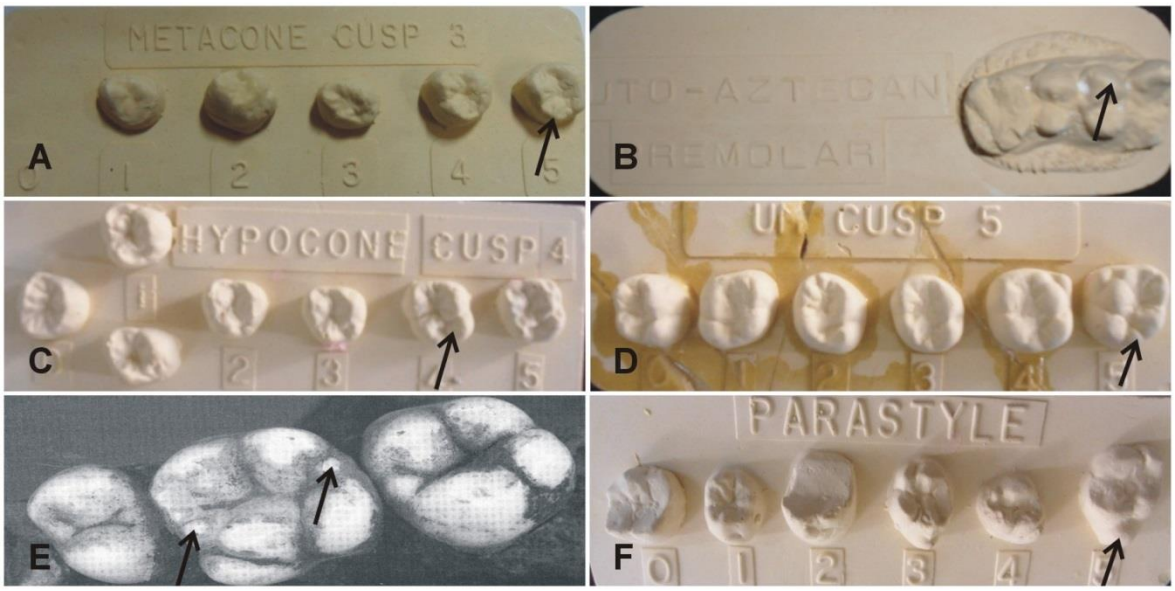


Fig.5. Scales for **A.** Metacone in upper molars **B**. UTO-Aztecan premolars **C**. Hypocone in upper molars **D**. Metaconule in upper molars **E**. Lower arrow shows protoconule in upper molars **F**. Parastyle in upper molars

2.2.15. Carabelli's trait

Carabelli's cusp is in fact only one of a group of features arising from the base of the mesiolingual cusp in upper molars (Hillson, 1996). A tubercle was checked on the mesiolingual aspect of the protocone (Cusp 2) and scored according to the standard scale in M1, M2 and M3 (Fig. 6A).

2.2.16. PEG

A Peg-Shaped Incisor (upper lateral incisor) is much reduced in size and lacks the normal morphology, being instead peg-shaped. It is probably related to congenital absence (Turner *et al*., 1991). Teeth reduced in size were checked for in the maxilla (M1, I2, P4) and in the mandible (M3, I2) and scored 1 if present (Fig. 6B).

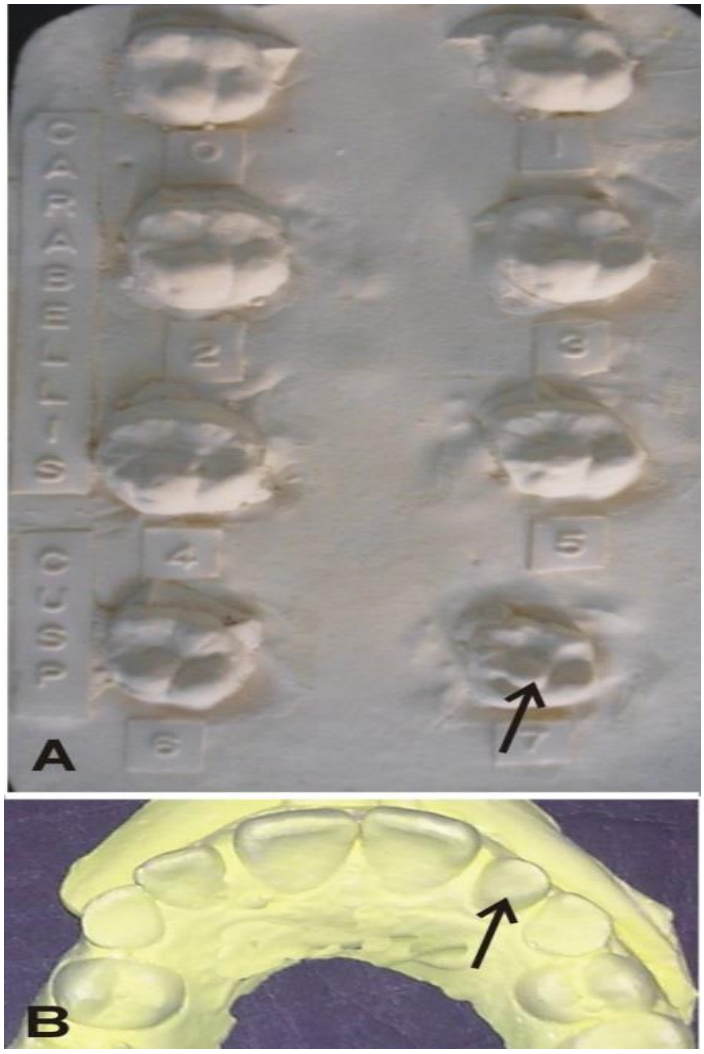


Fig.6. Scale showing **A**. Carabelli's trait in upper molars **B**. Arrow head point out PEG in upper lateral incisor.

2.3 Scoring Mandibular Traits

2.3.1. Mandibular Shoveling

Lingual shoveling of the lower incisors and canines was observed. The ridges and depth were checked and scored according to the ASUDAS lower incisors shoveling plaque (Fig. 7).

Fig.7. Scale for shoveling anterior in lower incisors and canines

2.3.2. Distal Accessory Ridges

The distal accessory ridge was studied on the distal side of the canine and scored according to the standard (Fig. 8A).

2.3.3. Premolar Accessory Cusps

Multiple lingual cusps are common in the lower premolars, which can exhibit one, two, three or more lingual cusps and cusplets (Scott and Turner, 1997). This trait has been

difficult to quantify because the lingual cusp is expressed in such a variety of forms. No standard scale available. It was studied on the lingual side of P3 & P4, if one cusp is being present the condition is labelled as "0" and if two cusps (one extra cusp) were present then scored 2 (there is no score of "1") and if 3 cusps are present, then score of 3 is assigned (Fig. 8B). If the normal cusp was absent then the condition labeled as "A" and it is an abnormality.

2.3.4 Premolar groove

No standard scale is available for comparison. The groove just at the lingual side of the P3 & P4 was studied; the groove seems to be like line going down from P3 & P4 towards the gum. If the groove was absent then scored 0, if was present at mesial side then scored 1, distal side scored 2, both sides scored 3 and scored 4 at the center (Fig. 8C).

2.3.5 Anterior Fovea

A fovea is located on the anterior occlusal surface of lower molars (Turner *et al*., 1991). This trait is among the most difficult to score (Scott and Turner, 1997). Because of rapid wear it is recommended that anterior fovea observations be limited in non-industrial individuals to those whose age is less than 12 years (Turner *et al.,* 1991). It was studied on the anterior occlusal surface in M1 only and scored according to the standard scale (Fig. 8D).

2.3.6 Y groove pattern

The groove pattern determines which cusps are in contact with each other (Turner *et al.,* 1991). The different patterns resemble the letters Y and X and the + mark. Pattern Y is formed when cusps 2 and 3 are in contact. Pattern X is formed when cusps 1 and 4 are in contact. A "plus" pattern is formed when all four main cusps are in contact (Turner *et al.,* 1991). Groove was studied only for Y pattern in our population in M1, M2 and M3 (Fig. 8E). The connection between cusp 2 and 3 was studied for Y pattern if it was present then scored 1 otherwise 0.

2.3.7 Major cusps number

Most permanent lower first molars have five cusps; mesiobuccal, mesiolingual, centrobuccal, distobuccal and distolingual, but there may be four or three. In four-cusped forms, the distobuccal cusp is missing, whilst the distolingual is additionally missing in three-cusped forms. Major cusps number was studied and scored according to the cusp numbers.

2.3.8 Deflecting Wrinkles

Deflecting wrinkle is defined by Scott *et al.* (1997), partially quoted from Weidenreich (1937) and Morris (1970) as follows: The median occlusal ridge of the metaconid often follows a straight course from the cusp tip to the central fossa. The median occlusal ridge of the metaconid was studied and scored by comparing with the standard scale in M1, M2 and M3 (Fig. 8F).

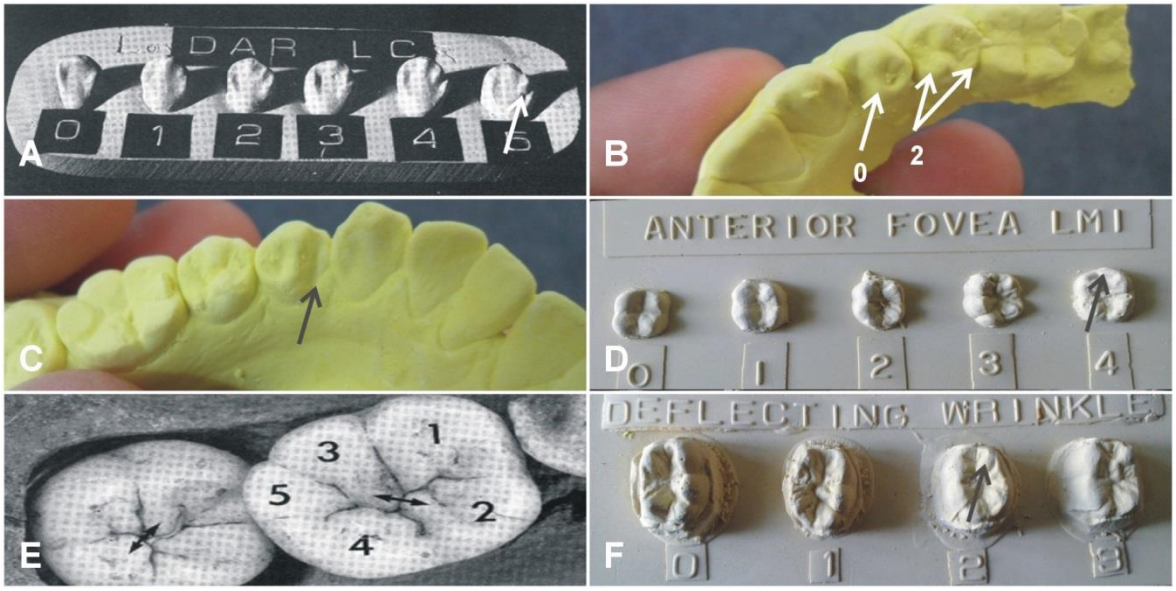


Fig.8. Scales for **A.** Distal accessory ridges in lower canines **B.** Premolar acccessory cusp 0 and 2 showing one and two cusps repectively in the lower premolars **C.** Arrow pointing out the groove in lower premolars **D.** Anterior fovea in lower molar 1 **E.** Arrows showing the connection b/w cusp 2 & 3 in lower molars **F.** Deflecting wrinkles in Iower molars

2.3.9 Protostylid

The protostylid is a paramolar cusp found on the buccal surface of cusp 1 (Turner *et al.,* 1991). An extra cusp or outgrowth was studied on the buccal surface of the protoconid (cusp 1) and scored according to the standard scale (Fig. 9A).

2.3.10 Cusp 5

Cusp 5, or the hypoconulid, occurs on the distal occlusal aspect of the lower molars. It is graded in terms of size only in the absence of cusp 6 (Turner *et al.,* 1991). Cusp 5, when it is present, is distal to cusp 3 and was scored according to the standard scale in M1, M2 and M3 (Fig. 9B).

2.3.11 Cusp 6

Cusp 6, the endoconulid or tuberculum sextum, occurs in the distal fovea of the lower molars lingual to cusp 5. It is scored by size relative to cusp 5 (Turner *et al*., 1991). When present, it was studied at its common location, lingual to cusp 5 (or between cusp 4 and cusp 5) and compared with the standard scale in M1, M2 and M3 (Fig. 9C).

2.3.12 Cusp 7

Cusp 7, the metaconulid or tuberculum intermedium, occurs in the lingual groove between cusps 2 and 4 of the lower molars, most commonly on the first molar (Turner *et al,*. 1991). Cusp 7 was scored according to the standard scale in M1, M2 and M3 (Fig. 9D).

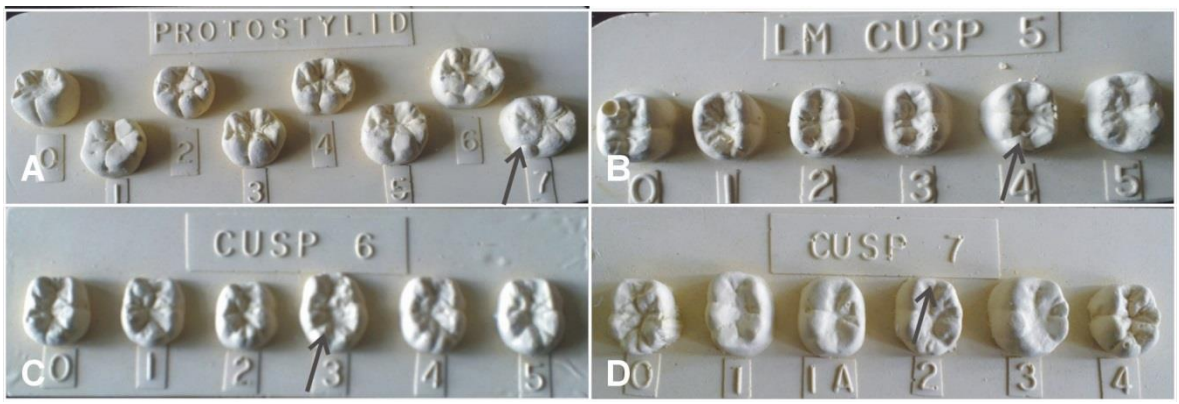


Fig.9. Scales for **A**. Protostylid in lower molars **B**. Cusp 5 in lower molars **C**. Cusp 6 in lower molars **D.** Cusp 7 in lower molars

2.3.13 Premolar Lingual Cusp fusion

No standard scale is available for LCF. A line joining two cusps from the lingual side of lower molars was studied in P3 and P4, if it was present then scored 1 otherwise scored 0.

2.4 DNA Analysis

2.4.1 Collection of Saliva

Saliva samples were collected from individuals of the selected seven ethnic groups (Abbassi, Awans, Gujars, Jadoons, Karlal, Syeds and Tanolis). Proper instructions were provided to the subjects. The subjects of this experiment were asked to vigorously rinse their mouth for 1:30 minutes and brush their teeth properly. Each subject was then provided with 2-3 mL of 5% sucrose solution after cleaning in the mouth cavity. They were advised to keep the sugar solution in mouth cavity for two to three minutes and then spit into the sterile small cups. The samples were stored at room temperature until return to the laboratory and stored at -20 °C before processing.

2.4.2 DNA Isolation

DNA isolation from saliva (mouth wash) was done with the modified protocol of Ralser *et al*., (2006) from buccal epithelial cells. All the stock solutions and reagents were prepared according to the procedures described in Appendix-III. Approximately 5ml of subject's mouthwash was collected in 15ml sterile tubes. About 1ml of liquid saliva was taken in 1.5ml eppendorf tube. 100µl of lysis solution (2ml lysis buffer + 10µl of Proteinase K + 3µl of marcaptoethanol) was added to saliva sample and was mixed well. Sample was then incubated at 56^{o}C for 1 hour and 30 minutes. After incubation 600µl of Phenol:Chloroform (1:1) solution was added and was incubated at once for 5 minutes at room temperature. Sample was then centrifuged at 10,000 rpm for 10 minutes and supernatant was transferred into a fresh tube very carefully. Equal volume of isopropanol was added to the subjected supernatant and was incubated at 20^{o}C for 20

minutes. Sample was centrifuged again at 10,000 rpm for 15 minutes and upper layer was discarded and pellet was washed with 70% ethanol. Ethanol was discarded after centrifugation at 8000 rpm for 5 minutes and pellet was air dried. 30µl of double distilled water was added to the dried DNA pellet and was incubated at 56^{o}C for 10 minutes. DNA quality and quantity was then checked by Agarose Gel Electrophoresis.

2.5 Gel Electrophoresis of DNA Samples

The purified DNA sample was analyzed on agarose gel electrophoresis for the quantity and quality of DNA. The agarose gel was prepared as follows:

One g of agarose in 100 mL of TAE-buffer (1% gel) was heated in a microwave oven for one minutes. The solution was cooled to 45 ^{o}C and 10 µL of ethidium bromide was added. The gel solution was loaded onto a gel caster, kept at room temperature until solidified. The comb was removed and the gel was placed in an electrophoresis apparatus containing 200 mL of TAE-buffer. Five µL of DNA sample, mixed with three µL DNA loading dye was applied to the agarose gel. The electrophoresis process was carried out at 80 volts for 10 min and then 100 volts until the dye front was 2-3 cm from the gel bottom. The presence and position of DNA bands was visualized and photographed.

2.6 Amplification of DNA Fragments

The extracted genomic DNA was used as a template for the PCR amplification of HVSI and HVSII regions of *mt*DNA. PCR was performed using Taq DNA polymerase to amplify 451bp-long fragments in the HVS1 region and the 563bp-long fragments in the

HVSII region (np15974-16425 and np07-569 respectively in the Anderson's reference sequence). Primers detail is given in Table 2. The components of reaction mixture used for polymerase chain reaction are given in Table 3.

Table 2: Information about the (*mt*DNA HV regions) Primers used during the present study

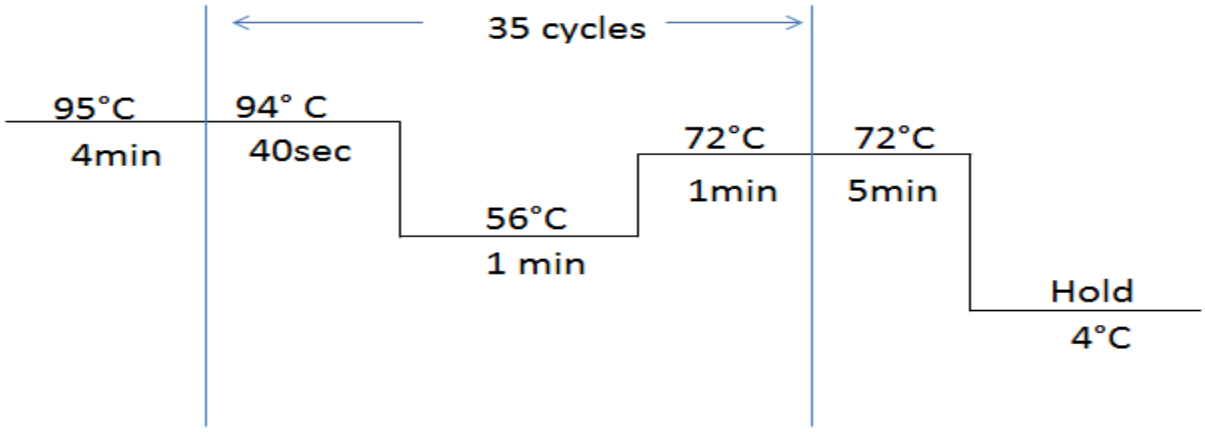
S.NO.	OLIGO NAME	SEQUENCE (5′ - 3′)	%GC	TM
1	HV1F	CTCCACCATTAGCACCCAAAGCTAAG	50	59.5
2	HV1R	GATATTGATTTCACGGAGGATGGTGGTC	46	59.9
3	HV2F	AGGTCTATCACCCTATTAACCACTCACG	46	60.0
4	HV2R	GGTGTCTTTGGGGTTTGGTTGGTTC	52	59.3

Table 3: Components of PCR reaction mixture

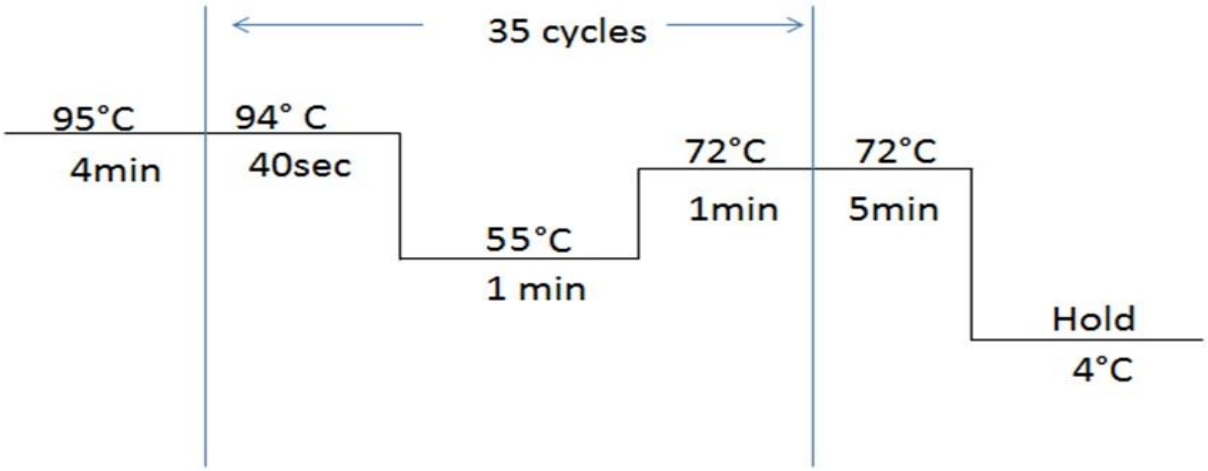
S. No.	Name of Reagent Added	Volume of Reagent	Final Concentration in the Reaction
1	10X Taq Buffer	2.5 µL	1X
2	2 mMdNTPs	2.0µL	0.16 mM
3	25mM $MgCl_2$	2.0µL	2.0 mM
4	10pM /µL F-Primer	2.0µL	20 pM
5	10 pM/µL R-Primer	2.0µL	20pM
6	Taq. Polymersae (5U/µL)	0.5µL	2.5 U
7	DNA template	2.0 µL	10-20 ng
8	ddH_2O	12 µL	12 µL
	Final Volume	25.0µL	

2.7 Conditions for PCR amplification

In general, PCR conditions for HVSI were: 95°C for 4min (initial denaturation), followed by 35 cycles of; 94 °C for 40 second, 56 °C for 1 min, and 72 °C for 1 min, with a final 5-min extension step at 72°C. And PCR conditions for HVSII were: 95°C for 4min (initial denaturation), followed by 35 cycles of; 94 °C for 40 second, 55 °C for 1 min, and 72 °C for 1 min, with a final 5-min extension step at 72°C. The annealing temperature was adjusted based on the Tm of the primers (Figure 10).



A.



B.

Fig.10. Sketch of the cycling profile of PCR, figures A and B reperesenting the optimized conditions for HVSI and HVSII respectively

The PCR product was then analyzed on 1.6% agrose gel. Amplified HVS bands were detected by placing the gel in UV tech gel documentation system and the bands were cutted and were processed further for cleaning of desired segment.

2.8 Elution of PCR Product

The gel containing PCR products excised from the gel with sterile blades and kept in labeled Eppendorf tubes and stored at -20^{o}C. The following procedure was adopted from GeneAll Gel Elution Kit (SV) Cat. no. 102-101 to isolate the product from the gel.

500µl of GB solution were added into the tube containing gel fragment of amplified HVS regions by PCR and were incubated at 60°c for 10 min until the gel was properly dissolved. The dissolved solution was shifted to VS column, was centrifuged at 13000 rpm/1min and liquid from the sink tube was discarded. After that 500 µl of wash buffer was added to the column and was centrifuged at 13000 for 2min. liquid in the sink tube was removed and the VS column was centrifuged again for 2 minutes to complete washing of PCR product. At the end the column was shifted to a fresh eppendorf tube and 50 µl of water was added and was incubated at 60°C for 2 min and were then kept at room temperature for 5 minute and finally was centrifuged at 13000 rpm for 2 minutes. And isolated PCR product was then checked on agarose gel electrophoresis.

The purified PCR product was sent to Macrogen Inc. Korea for sequence analysis. Sequencing was performed using the BigDye Terminator Cycle Sequencing Kit (AB) and sequences were analyzed on a 3730 Genetic Analyzer (AB).

2.9 Statistical Analysis

The data recorded for dental trait frequencies were analyzed by Hierarchical Cluster Analysis with Ward's Linkage, Neighbor-joining Cluster Analysis, Multidimensional Scaling (MDS)—Kruskal's Method, Multidimensional Scaling (MDS)—Guttman's Method, Principal Coordinates Analysis (PCO) and Diagonal matrix of Smith's Mean Measure of Distances between sample pairs with both living and prehistoric samples and living northern Pakistani groups only.

The nucleotide sequences data obtained from *mt*DNA were aligned and reported with respect to the revised version of the Cambridge reference sequence (Anderson, *et al*., 1981; Andrews, *et al*., 1999).

All the HV1 and HVII sequences were then investigated for finding haplotypes using the online database MTHAP (http://dna.jameslick.com/mthap/). All the haplotypes were then tabulated and compared with other reported haplotypes representing different ethnic groups of Pakistan. Sequences were aligned using BioEdit (Hall, 1999). All the sequences were scrutinized performing a quasi-median network analysis and using the software Network, available on the EMPOP website (Parsona and Dürb, 2007; Parson, *et al*., 2004).

Chapter-3
RESULTS

3.1 Dental Morphology

Table 4 lists the maximum number of individuals for which dental data were collected from the tribes in District Abbottabad and Mansehra and for comparing living and ancient regional populations. Analyses of the data and their comparison through hierarchical cluster analysis, neighbor-joining cluster analysis, multidimensional scaling with both Kruskal's method and Guttman's methods, and principal coordinates analysis, provide the following description of the results with respect to living and ancient people of the region.

3.1.1 The Living Populations

Mean measure of divergence (MMD) analysis of the patterns of phenetic affinities possessed between all the selected tribes of Abbottabad and Mansehra and their standardized pairwise distances are provided in Table 5. The distance matrix values were then used to create the basis for the remaining analyses. It should be noted that all negative values within the distance matrix were reset to 0 prior to submission to further analytical procedures. Values closest to zero and below indicate a close relationship between the compared samples.

Table 4: Details of the maximum number of individuals possible to score

Sample	Abb.	n_{max}[1]		Sample	Abb.	n_{max}[1]
Northern Pakistan				**Prehistoric Central Asia**		
Awans	AWA1	167		Sapalli Tepe	SAP	43
Khowars	KHO	144		Djarkutan	DJR	39
Madaklasht	MDK	185		Kuzali	KUZ	24
Swatis	SWT	178		Molali	MOL	41
Wakhis (Gulmit)	WAKg	162		**Prehistoric Indus valley**		
Wakhis (Sost)	WAKs	146		Neo. Mehrgarh	NeoMRG	49
Abbottabad and Mansehra				Chl. Mehrgarh	ChlMRG	25
Awans	AWA2	93		Harappa	HAR	33
Syeds	SYD	65		Timargarha	TMG	25
Gujars	GUJ	90		Sara i Khola	SKH	15
Tanolis	TAN	69		**South-Eastern Indians**		
Karlals	KAR	76		Pakanati Red.	PNT	182
Western Indians				Gompad. Mad.	GPD	178
Inamgaon	INM	41		Chenchus	CHU	194
Marathas	MRT	198				
Mahars	MHR	195				
Madia Gonds	MDA	169				

Total = 2,826 (2,433 + 393)

1. **= n_{max} is the maximum number of individuals possible to score for those tooth-trait combinations considered.**

Table 5: Mean Measure of Divergence Analysis of the living population of Northern Pakistan

	AWAm1	AWAm2	GUJm2	KARa	KHO	MDK	SWT	SYDm2	TANM2	WAKg	WAKs
AWAm1	….	0.005	0.005	0.006	0.006	0.004	0.005	0.005	0.005	0.005	0.005
AWAm2	0.006	....	0.006	0.006	0.006	0.005	0.005	0.006	0.006	0.005	0.005
GUJm2	0.046	0.069	….	0.006	0.007	0.005	0.005	0.006	0.006	0.006	0.005
KARa	0.035	0.085	0.017	….	0.007	0.005	0.005	0.006	0.006	0.006	0.006
KHO	-0.008	-0.004	0.053	0.051	….	0.006	0.006	0.007	0.007	0.006	0.006
MDK	0.004	0.039	0.049	0.039	0.013	….	0.004	0.005	0.005	0.005	0.005
SWT	0.003	0.034	0.078	0.056	0.006	-0.002	….	0.005	0.005	0.030	0.029
SYDm2	0.016	0.024	-0.001	0.019	0.019	0.032	0.044	….	0.006	0.006	0.006
TANm2	0.012	-0.003	0.040	0.068	0.009	0.043	0.041	0.002	…	0.006	0.006
WAKg	0.000	0.009	0.066	0.061	0.001	0.005	0.003	0.030	0.023	….	0.005
WAKs	0.009	0.005	0.077	0.084	0.007	0.016	0.020	0.040	0.027	-0.007	….
MMD= Below Diagonal											
MMDsd= Above Diagonal											

Hierarchical cluster analysis was used to determine which groups are most similar to one another and which are the least similar. Hierarchical cluster analyses were based upon Euclidean distances and linkages between sample pairs, were made in accordance with Ward's (1963) Minimum Variance technique. Hierarchical cluster analysis with Ward's linage yielded a fundamental split between Syeds, Gujars and Karlals, versus all other groups. Among all the tribes outliers, affinities were close between Syeds and Gujars, while affinities with Karlals to both of these tribes were closer as compared to others. In the other set of samples, Awans and Tanolis samples collected from Mansehra were peripheral outliers (unpublished report of UCD) to the Madaklasht tribe of Chitral, Swatis of Mansehra District, Khowars of Chitral District. Other sample of Awans from Mansehra and the two geographically distinct samples of Wakhis from Gilgit-Baltistan clustered closely. These cluster analysis suggests the two Wakhi samples have closely related to one another, and are distantly related to the Khowars and Awans from Mansehra. The Madaklasht tribe of Chitral and the Swatis from Mansehra has close affinities to one another. The cluster analysis report is provided in Figure 11.

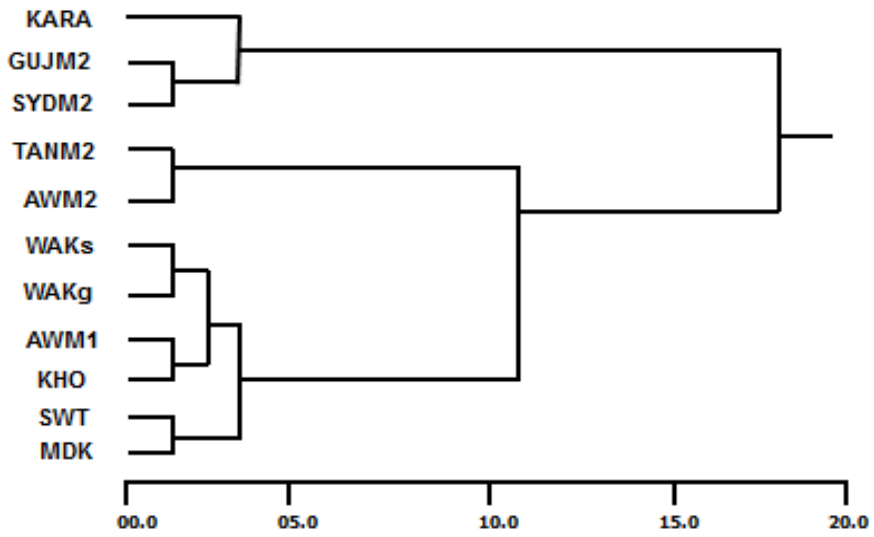


Fig.11. Cluster analysis of living populations of Northern Pakistan showing a fundamental split between Syeds (SYDM2), Gujars (GUJM2) and Karlals (KARA), versus all other groups

Results of the Neighbor-joining analysis were similar to hierarchical cluster analysis but have an advantage and that is, the Neighbor-joining analysis allows lineages (similarities) to vary at unequal rates and is suitable for depicting linkages among samples with varying degrees of affinity to one another. Results of the Neighbor-joining cluster analysis are given in Figure 12. The Neighbor-joining analysis agrees with most, but not all of the affinities identified by hierarchical cluster analysis. The distinctiveness of the Karlals, Gujars and Syeds from the other samples is reflected by their segregation on the left side (Fig. 12). However, N-J cluster analysis indicates that affinities are closer between Karlals and Gujars, than either ethnic groups shares with Syeds. Syeds have equally distant affinities to Karlals-Gujars on the one hand and Tanolis from Mansehra on this other. Tanolis share closest affinities with Awans from Mansehra District but

interestingly the two samples of Awans do not match with one another with respect to affinities. Rather Awan 2 closely resembles Khowars of Chitral District. It is a general observation that the people with close affinities though separated by lines or boundaries e.g. the two geographically distinct samples of Wakhis (WAKs and WAKg) retain the genetic relationship and segregate accordingly. Similarly the Madaklasht and Swatis show closest affinities to one another and have only distant affinities to the other samples included in the analysis. Their only affinities lie with the sample of Awans from Mansehra District collected by Hemphill near Baffa.

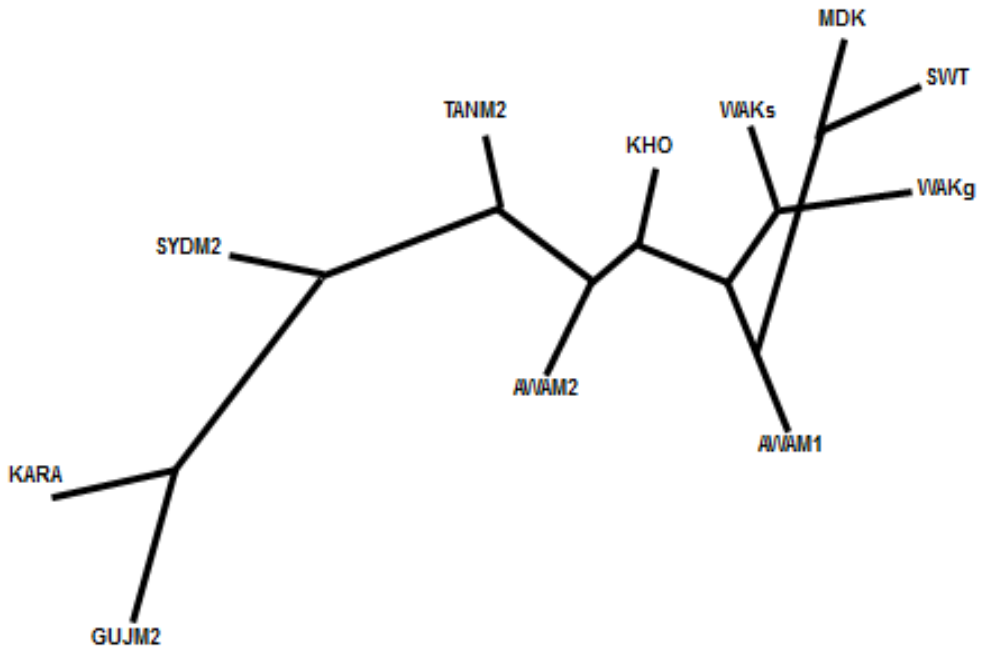


Fig.12. Neighbor-joining analysis of living populations of Northern Pakistan indicating segregation of Syeds (SYDM2), Gujars (GUJM2) and Karlals (KARA) on the left side

The multidimensional scaling provides information regarding the degree of association between one or more objects, similar to the Neighbor-Joining analysis, but is based on several variables. There are several types of multidimensional scaling, but Guttman's (Fig. 14) and Kruskal's (Fig. 13) methods were used for elaborating the degree of association between different tribes of Mansehra and Abbottabad.

The multidimensional scaling into three dimensions with Kruskal's method (Fig. 13) was accomplished in 133 iterations, with a stress value of 0.011 (extremely good fit) accounting for 99.9% of the variance between samples. Segregated from all other samples on the left side of the plot, MDS confirms the distinctiveness of the Syeds, Gujars and Karlals relative to the other samples, as has been identified previously from hierarchical cluster analysis (Fig. 11) and neighbor-joining cluster analysis (Fig. 12). As seen in the neighbor-joining cluster analysis, it is the Tanolis that link them to the remaining tribes through Awans from Mansehra District. Once again, the two samples of Awans do not exhibit particularly close affinities to one another, for the sample of Khowars from Chitral District is interposed in between them in multidimensional space. The sample of Awans 1 collected near HU campus and in Baffa has distant affinities to the two samples of Wakhis—who show close affinities to one another—on the one hand, and to Swatis and the Madaklast on the other. In a departure from neighbor-joining cluster analysis, it is clear that Swatis are more similar to Awans 1 than are the Madaklasht, who are identified as possessing no close affinities to any of the other samples included in this analysis, including the Swatis from Mansehra District.

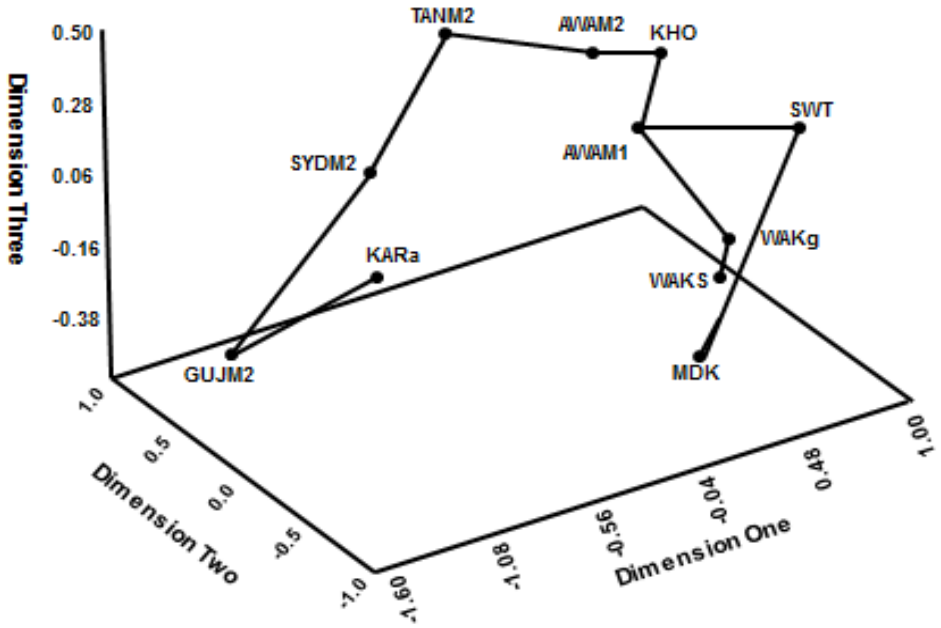


Fig.13. Multidimensional scaling through Kruskal's multivariate analysis of the tribes from Mansehra and Abbottabad confirms the distinctiveness of the Syeds (SYDM2), Gujars (GUJM2) and Karlals (KARA) relative to the other samples

The multidimensional scaling into three dimensions with Guttman's method (Fig. 14) was accomplished in 17 iterations, with a stress value of 0.037 (extremely good fit) accounting for 99.56% of the variance between samples. Once again, the Karlals, Gujars and Syeds are segregated away from all other samples on the left side of the array. While Kruskal's method suggests all three samples are equidistant from one another with regard to genetic distance, Guttman's method indicate affinties are slightly closer between Gujars and Syeds, while the Karlals are identified as an outlier with no close affinities to the other tribes, except for the Gujars. Once again, Tanolis from Mansehra District are identified as a sort of "bridge" linking these three divergent groups to the

other northern Pakistani tribes and the two populations of Awans do not show particularly close affinities to one another, for the tribe of Khowars from Chitral District are interposed in between them. As evidenced in the MDS plot with Kurskal's methods, MDS with Guttman's method shows that Awans of Mansehra have equally distant affinities to the two Wakhi samples (who have close affinities to one another) and to Swatis from Mansehra District. As was the case in the MDS plot with Kruskal's methods, the MDS plot with Guttman's method indicates that the Madaklasht are divergent from all other samples, and share only distant affinities to the Swatis from Mansehra District.

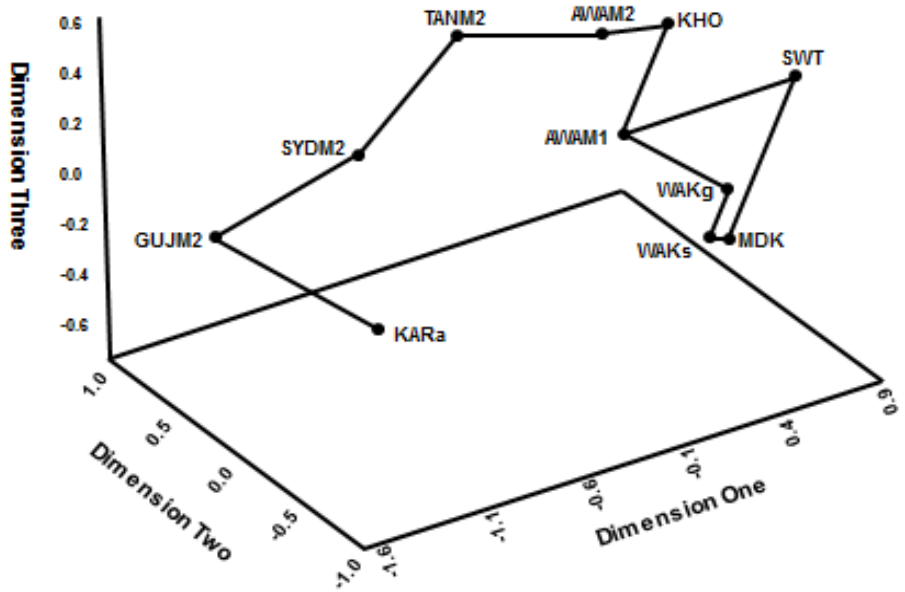


Fig.14. Multidimensional scaling of the people of central Hazara through Guttman's method presenting segregation of Karlals, Gujars and Syeds away from all other samples on the left side

Results obtained through Principal Coordinate Analysis (PCO) show that first three principal axes generated by PCO capture 95.6% of the total variance among samples (Fig. 15). This plot shows many similarities, but also generates some differences from the cluster analyses and multidimensional scaling results. Like all other analyses the plot of the first three principal axes identifies Karlals, Gujars and Syeds as divergent from the remaining samples. Karlals are clearly the most divergent of the three. Like MDS with Kruskal's method these three samples appear to be equidistant from one another phenetically and Tanolis link these three samples with the other samples. However, in a bit of a departure from the results obtained by MDS, but in agreement with neighbor-joining cluster analysis, Tanolis are identified as possessing closer affinities to Awans from Mansehra District than Tanolis possess to Syeds. In another departure, PCO does not identify any particularly close affinity between Awans 2 and Khowars. This is very different from the MDS plot generated by Kruskal's method and by neighbor-joining cluster analysis. Instead, affinities with Khowars are much, much closer with the sample of Awans 1. This is a result not found in any of the other analyses. The two MDS plots, PCO identifies Awans 1 as possessing affinities to the two geographically distinct samples of Wakhis on the one hand and to Swatis and the Madaklasht on the other. As it is visible in the case in both MDS plots, affinities are closer with the sample of Wakhis from Gulmit (WAKg) than with the more northerly sample of Wakhis from Sost (WAKs). This relationship is reversed in the neighbor-joining cluster analysis. In yet another unique result, PCO suggests that the sample of

Awans 1 have close affinities with the sample of Swatis of Mansehra District, yet like the two MDS plots, it is clear the Madaklasht are more divergent.

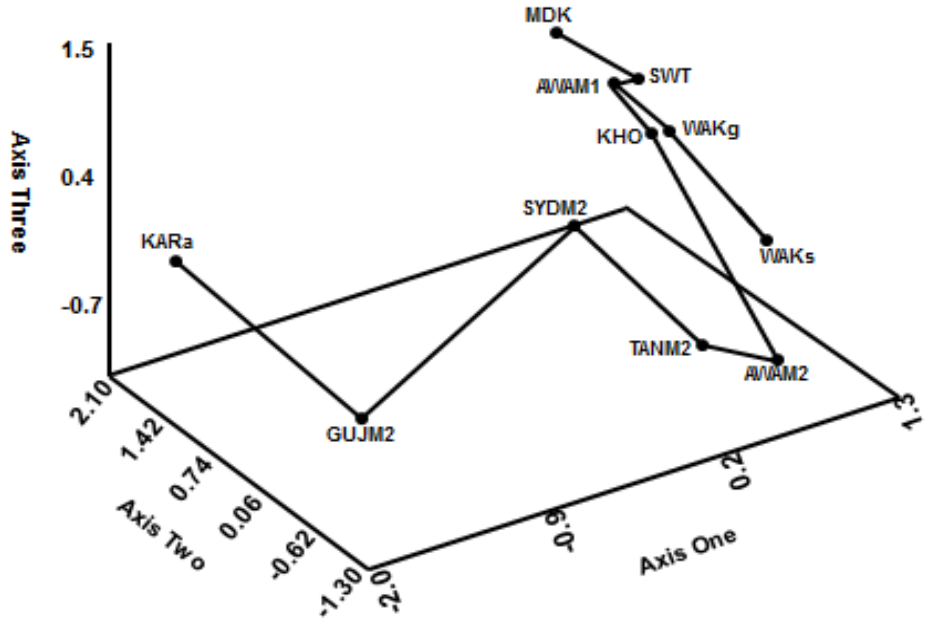


Fig.15. Principal Coordinates analysis of the results obtained for different populations displaying divergence of Karlals, Gujars and Syeds from all other samples

3.1.2 Living VS Ancient People of the Region

Mean measure of divergence (MMD) analysis was conducted for determination of the patterns of phenetic affinities between living Pakistanis considered in light of living Peninsular Indians and prehistoric inhabitants of the Indus Valley and south-central Asia. The pairwise standardized distances are provided in Table 6.

Table 6: Mean Measure of Divergence Analysis of the populations under study

	AWAm1	AWAm2	ChlMRG	CHU	DJR	GPD	GUJm2	HAR	INM	KARa	KHO	KUZ	MDK	MDA	MHR	MRT	MOL	NeoMRG	PNT	SAP	SKH	SWT	SYDm2	TANm2	TMG	WAKg	WAKs
AWAm1	…	0.005	0.018	0.004	0.016	0.004	0.005	0.023	0.015	0.006	0.006	0.023	0.004	0.005	0.004	0.004	0.013	0.013	0.004	0.015	0.032	0.005	0.005	0.005	0.029	0.005	0.005
AWAm2	0.006	…	0.018	0.005	0.016	0.005	0.006	0.023	0.015	0.006	0.006	0.024	0.005	0.005	0.005	0.005	0.013	0.013	0.005	0.016	0.033	0.005	0.006	0.006	0.03	0.005	0.005
ChlMRG	0.069	0.154	…	0.017	0.029	0.018	0.019	0.036	0.026	0.019	0.019	0.036	0.018	0.018	0.022	0.018	0.026	0.026	0.018	0.028	0.045	0.018	0.019	0.019	0.042	0.018	0.018
CHU	0.052	0.07	0.049	…	0.016	0.004	0.005	0.022	0.015	0.005	0.005	0.023	0.004	0.004	0.004	0.004	0.012	0.012	0.004	0.015	0.032	0.004	0.0005	0.005	0.029	0.005	0.004
DJR	0.13	0.132	0.102	0.079	…	0.016	0.017	0.034	0.026	0.017	0.017	0.035	0.015	0.016	0.016	0.016	0.024	0.024	0.016	0.027	0.044	0.016	0.017	0.097	0.04	0.016	0.016
GPD	0.051	0.079	0.071	0.005	0.13	…	0.005	0.022	0.015	0.005	0.006	0.023	0.004	0.004	0.004	0.004	0.013	0.012	0.004	0.015	0.032	0.004	0.005	0.005	0.029	0.005	0.005
GUJm2	0.046	0.069	0.079	0.103	0.174	0.119	…	0.023	0.016	0.026	0.007	0.024	0.005	0.005	0.005	0.005	0.013	0.013	0.005	0.016	0.033	0.005	0.006	0.006	0.03	0.006	0.005
HAR	0.022	0.032	0.05	0.049	0.139	0.058	0.102	…	0.033	0.023	0.024	0.041	0.023	0.023	0.022	0.022	0.031	0.31	0.022	0.033	0.05	0.023	0.023	0.023	0.047	0.023	0.023
INM	0.027	0.056	0.116	0.076	0.127	0.074	0.08	0.039	…	0.016	0.016	0.034	0.015	0.015	0.015	0.015	0.023	0.023	0.015	0.026	0.042	0.015	0.016	0.016	0.04	0.016	0.015
KARa	0.035	0.085	0.051	0.097	0.17	0.084	0.017	0.074	0.061	…	0.007	0.024	0.005	0.005	0.005	0.005	0.014	0.013	0.005	0.016	0.033	0.005	0.006	0.006	0.03	0.006	0.006
KHO	-0.006	-0.004	0.078	0.036	0.111	0.047	0.053	0.027	0.042	0.051	…	0.024	0.006	0.006	0.006	0.005	0.014	0.014	0.006	0.016	0.033	0.006	0.007	0.007	0.03	0.006	0.006
KUZ	0.125	0.121	0.101	0.1	-0.044	0.143	0.154	0.075	0.119	0.146	0.123	…	0.023	0.023	0.023	0.023	0.031	0.031	0.023	0.034	0.05	0.023	0.024	0.024	0.047	0.024	0.023
MDK	0.004	0.03	0.093	0.076	0.208	0.061	0.049	0.051	0.068	0.039	0.013	0.19	…	0.004	0.004	0.004	0.013	0.012	0.004	0.015	0.032	0.004	0.005	0.005	0.029	0.005	0.005
MDA	0.038	0.053	0.104	0.042	0.152	0.036	0.082	0.049	0.065	0.063	0.043	0.166	0.065	…	0.004	0.004	0.013	0.013	0.004	0.015	0.032	0.004	0.005	0.005	0.029	0.005	0,005
MHR	0.017	0.036	0.104	0.061	0.228	0.051	0.056	0.026	0.028	0.055	0.021	0.221	0.023	0.019	…	0.004	0.013	0.012	0.004	0.015	0.032	0.004	0.005	0.005	0.029	0.005	0.005
MRT	0.021	0.039	0.107	0.006	0.185	0.045	0.06	0.015	0.005	0.048	0.032	0.166	0.041	0.005	0.002	…	0.021	0.012	0.004	0.015	0.032	0.004	0.005	0.005	0.029	0.005	0.004
MO	0.076	0.08	0.021	0.0	-	0.0	0.1	0.0	0.11	0.1	0.0	-	0.1	0.1	0.1	0.1	…	0.01	0.01	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

L		3		48	0.026	96	1	49	4	14	55	0.033	21	35	51	4		2	3	24	4	13	14	14	37	13	13
NeoMRG	0.091	0.157	0.061	0.113	0.167	0.118	0.102	0.064	-0.004	0.071	0.109	0.176	0.12	0.036	0.064	0.057	0.153	…	0.012	0.023	0.04	0.012	0.013	0.013	0.037	0.013	0.013
PNT	0.034	0.067	0.076	0.028	0.19	0.008	0.121	0.027	0.062	0.081	0.035	0.189	0.041	0.051	0.028	0.033	0.128	0.127	…	0.015	0.032	0.004	0.005	0.005	0.029	0.005	0.005
SAP	0.115	0.101	0.065	0.069	-0.044	0.042	0.152	0.09	0.146	0.061	0.087	-0.053	0.177	0.163	0.195	0.171	-0.051	0.207	0.163	…	0.043	0.015	0.016	0.016	0.04	0.016	0.015
SKH	0.082	0.061	0.148	0.072	0.085	0.097	0.127	0.026	-0.013	0.144	0.08	-0.015	0.158	0.058	0.095	0.041	0.062	0.096	0.118	0.039	…	0.032	0.033	0.033	0.056	0.033	0.032
SWT	0.003	0.034	0.074	0.049	0.172	0.076	0.078	0.025	0.074	0.055	0.006	0.155	0.002	0.066	0.025	0.045	0.09	0.123	0.019	0.141	0.15	…	0.005	0.005	0.029	0.03	0.029
SYDm2	0.016	0.024	0.078	0.062	0.127	0.066	-0.001	0.053	0.067	0.019	0.019	0.111	0.032	0.045	0.039	0.041	0.068	0.117	0.075	0.103	0.094	0.044	…	0.006	0.03	0.006	0.006
TANm2	0.012	-0.003	0.121	0.051	0.097	0.056	0.04	0.05	0.064	0.068	0.009	0.083	0.043	0.05	0.044	0.043	0.055	0.156	0.068	0.07	0.049	0.041	0.002	…	0.03	0.006	0.006
TMG	-0.002	0.003	0.081	0.038	0.083	0.056	0.007	-0.001	-0.053	0.021	0.001	0.041	0.039	0.006	0.01	-0.009	0.043	0.045	0.062	0.071	-0.077	0.056	-0.014	-0.01	…	0.005	0.005
WAKg	0	0.009	0.107	0.066	0.172	0.067	0.056	0	0.043	0.061	0.001	0.143	0.005	0.059	0.017	0.032	0.066	0.122	0.045	0.131	0.096	0.003	0.03	0.023	0.006	…	0.005
WAKs	0.009	0.005	0.154	0.091	0.209	0.095	0.077	0.018	0.059	0.064	0.007	0.173	0.016	0.073	0.028	0.044	0.119	0.154	0.065	0.164	0.099	0.02	0.04	0.027	0.008	-0.007	…
MMD= Below Diagonal																											
MMDsd= Above Diagonal																											

Hierarchical cluster analysis draws a fundamental distinction between the prehistoric inhabitants of south-central Asia (SAP, KUZ, DJR and MOL) and all other samples, both living and the prehistoric. Remaining samples fall into two aggregates—living ethnic groups of northern Pakistan versus prehistoric inhabitants of the Indus Valley and living ethnic groups of peninsular India (Fig. 16). Beginning with the latter aggregate, the three living Dravidian-speaking ethnic groups from southeastern India (PNT, GRP, CHU) exhibit closest affinities to one another and more distant affinity to the Chalcolithic era (c. 4500 B.C.) inhabitants of Mehargarh, a site located on the North Kachi Plain of Pakistan, near the modern town of Sibi. The other aggregate is composed of one group that includes the three living Indo-Aryan-speaking ethnic groups from Maharashtra (MDA, MRT, MHR) and the post-early Chalcolithic prehistoric samples from the Indus Valley (HAR, TMG, SKH) versus the Late Jorwe period sample from Inamgaon (c. 1400 B.C., west-central India) and the aceramic Neolithic period sample (c. 6000 B.C.) from Mehrgarh. What is reassuring about these results is that there is no simple segregation between living samples on the one hand and prehistoric samples on the other. This indicates that there is no systemic bias introduced when one mixes ancient samples with samples of living individuals.

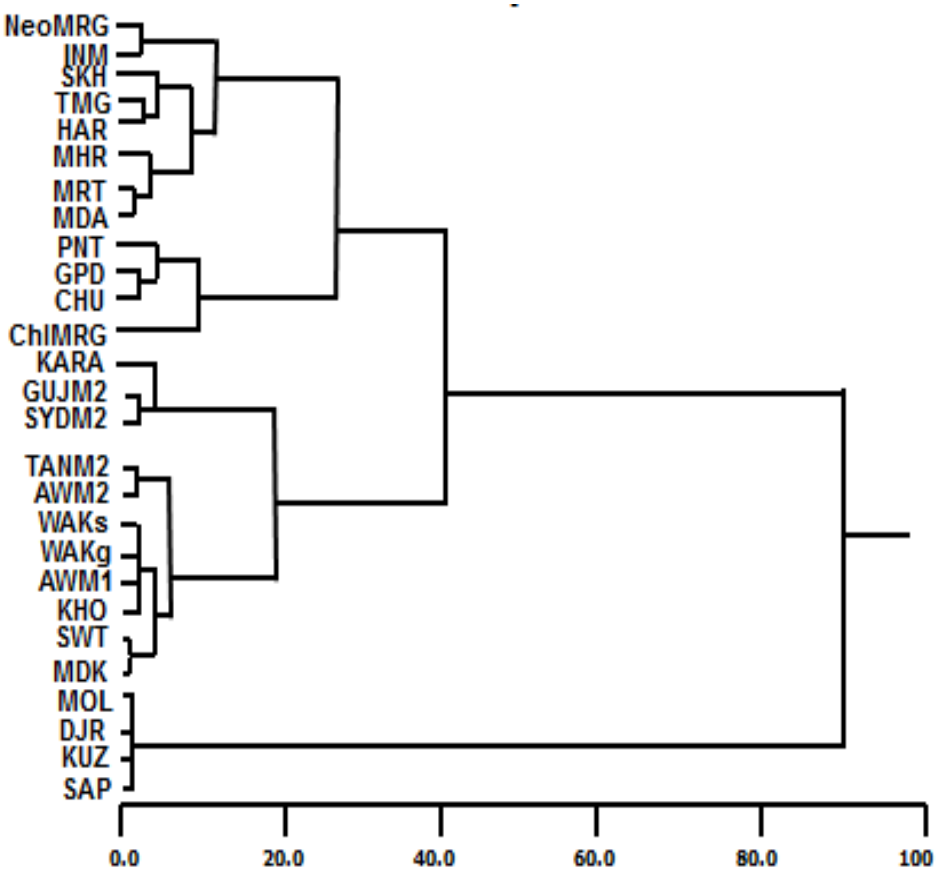


Fig.16. Cluster analysis of the living Pakistani with other living and prehistoric people of the region

Neighbor-joining cluster analysis identifies four sample aggregates (Fig. 17). These are located in the lower left, upper left, upper center, and the lower right. In the lower left may be found the four prehistoric samples from south-central Asia, with the Chalcolithic sample from Mehrgarh joining them at a more distant remove. This mirrors the results described for hierarchical analysis described above. The second aggregate is composed of the three living samples of Dravidian-speaking groups from southeastern India (GPD, PNT and CHU). Interestingly, the Mature Phase (2300-1800 B.C.) sample from Harappa is identified as possessing nearly equidistant affinities to these

prehistoric south-central Asians and living Dravidian-speakers of southeastern India. The aggregate in the upper center includes the remaining prehistoric samples from the Indus Valley, the prehistoric sample from west-central India (INM), as well as two of the three living ethnic groups from Maharashtra (MDA, MRT). The affinity between NeoMRG and INM has shown up a number of times and doesn't seem odd. The affinity between these prehistoric inhabitants of the Indus Valley and living ethnic groups of west-central India is unique. The third of the living Indo-Aryan-speaking groups, the low-status Mahars, are identified as possessing equidistant affinities between these prehistoric inhabitants of the Indus Valley and the two other living samples from west-central India (MDA, MHR) and an aggregate composed of living northern Pakistanis. The aggregate in the lower right encompasses the samples of living northern Pakistanis. This aggregate can be subdivided between highly divergent branches than encompasses the Syeds, Gujars and Karlals, and a second aggregate that includes all other samples of northern Pakistanis. Intriguingly it is Gujars, rather than Karlals who are identified as most divergent, while Syeds are most proximate (but only relatively) to members of the other aggregate of northern Pakistanis. The inhabitants of Madaklasht and Swatis are identified as possessing closest affinities to one another, and to the two geographically distinct samples of Wakhis who share closest affinities to one another. As it is evident in the comparison of living northern Pakistan only, it is the sample of Awans Hemphill collected from Mansehra (AWAm1) that links Wakhis, Swatis and the Madaklasht to Khowars, the sample of Awans analyzed in the present study (AWAm2) and Tanolis.

Intriguingly, Tanolis are identified as rather unique, and this is very different from their phenetic affinities when only northern Pakistani groups are included.

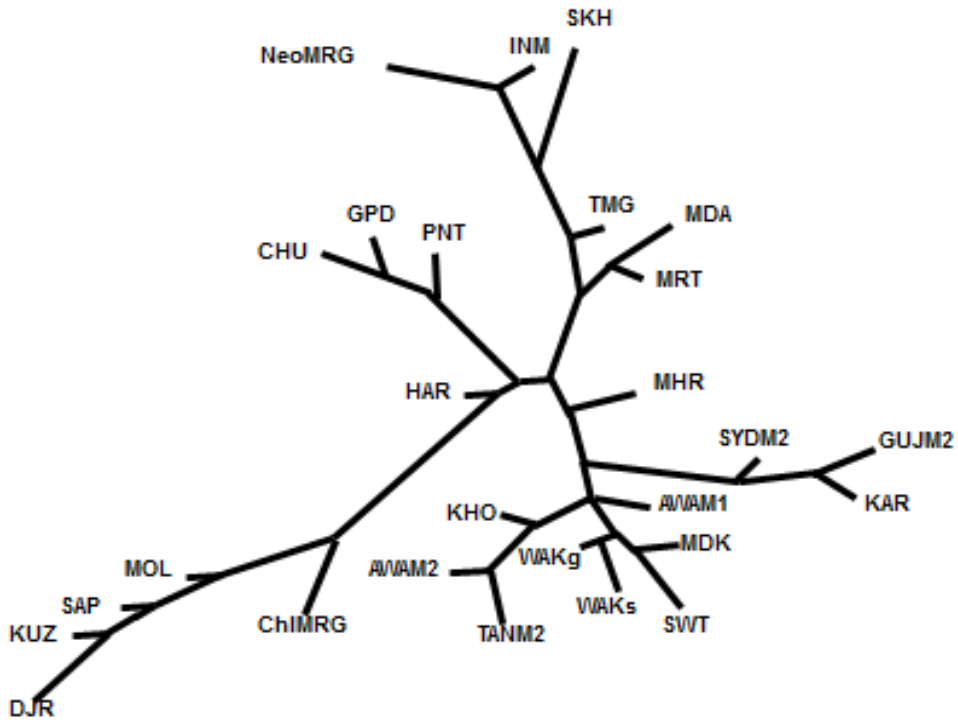


Fig.17. Neighbor-joining analysis of living Pakistani compared with the available information with other living and ancient people of the area

The multidimensional scaling into three dimensions with Kruskal's method was accomplished in 31 iterations, with a stress value of 0.132 (moderately good fit) accounting for 87.7% of the variance between samples (Fig. 18), isolated on the left side of the plot, the four prehistoric samples from south-central Asia clearly stand apart from all other samples. They connect to the remaining samples via a very long link with the latest of the prehistoric Indus Valley samples (SKH). Both, the Chalcolithic period sample from Mehrgarh and the sample of Chenchus, a living Dravidian-speaking tribal

population from southeast India, stand apart as outliers with no close affinities to any of the other samples included in this analysis. Remaining samples fall into two aggregates. The first is found in the upper right and includes the three living Indo-Aryan-speaking samples from Maharashtra, the two Dravidian-speaking caste samples from southeast India (which show no affinities to one another) and all of the prehistoric samples from the Indus Valley, except for the Chalcolithic inhabitants of Mehrgarh (an isolate) and the Iron Age inhabitants of Sarai Khola (also an isolate). The second aggregate is found in the lower right. It connects to other samples via the sample of living Khoars from Chitral District. Members of this aggregate are divided into two groups. The first includes Tanolis, Syeds, Gujars and Karlals. As we seen in the analysis that was limited to living northern Pakistanis only, Tanolis once again serve as a "bridge" between these samples and other northern Pakistanis, while Karlals stand out as most divergent. The other groups includes the two Awan samples, which show closest affinities to one another, the two Wakhi samples, which again show closest affinities to one another, and the two highly divergent samples of Swatis and Madaklasht. This plot suggests close affinities between the Madaklasht and Swatis.

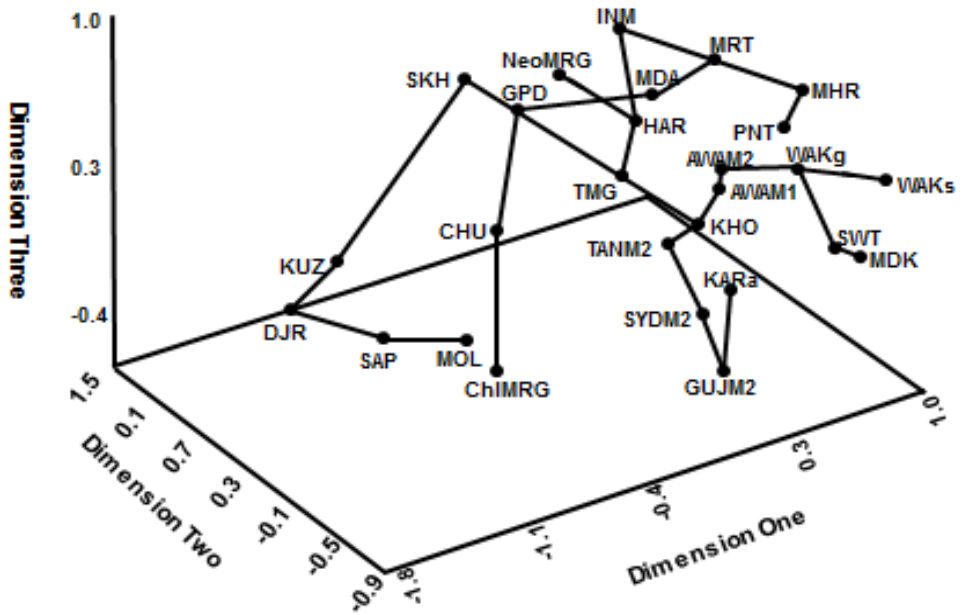


Fig.18. Multidimensional scaling through Kruskal's multivariate analysis

Multidimensional scaling into three dimensions with Guttman's method was accomplished in 31 iterations, with a stress value of 0.143 (moderately good fit) accounting for 88.7% of the variance between samples (Fig. 19). Once again, the four prehistoric south-central Asian samples are isolated on the left side of the array. This time, the latest of the prehistoric Indus Valley samples (SKH) is identified as possessing closer affinities to these south-central Asian samples than to earlier samples from the Indus Valley. Timargarha (TMG) and Harappa (HAR) share fairly close affinity in the center of the array. The Chalcolithic (ChlMRG) and Neolithic (NeoMRG) inhabitants of Mehargarh are both identified as outliers with little affinity to one another or to the other samples included in this analysis. Syeds, Gujars and Karlals are found in the upper center of the array. Karlals are identified as the most divergent, while Syeds link to other northern Pakistani groups via Tanolis, who share affinities with Awans from

Mansehra District. Once again the two samples of Awans are not particular close phenetically and Khowars are interposed in between them. Remaining northern Pakistani samples are found in the upper right background. Affinities are close between the two Wakhi samples, while the Madaklasht and Swatis are more divergent and show little affinity to one another. Peninsular Indian samples are found in the upper right foreground. Affinity is close between the three Indo-Aryan-speaking groups from Maharashtra, especially Marathas (MRT) and Madia Gonds (MDA). Affinities between the three Dravidian-speaking samples are not as close, but appear closer between the two Hindu caste samples (PNT, GPD) than with the tribal samples of Chenchus.

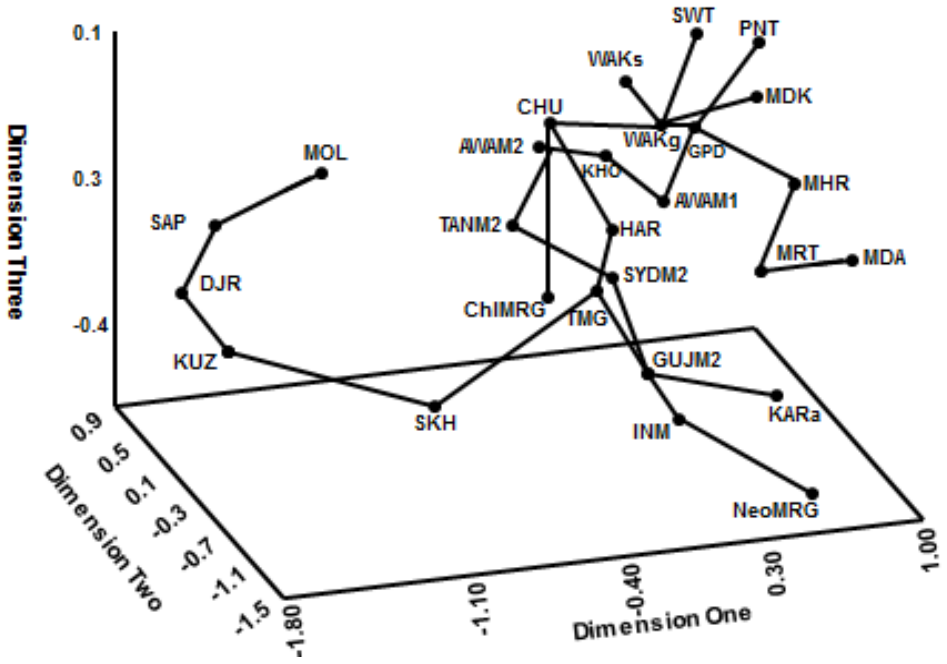


Fig.19. Multidimensional scaling through Guttman's method

The first three principal axes generated by principal coordinate analysis (PCO) capture 73.5% of the total variance among samples (Fig. 20). This plot shows many similarities, but also some differences from the cluster analyses and multidimensional scaling results. As in the other analyses, the four samples from south-central Asia are separated from all South Asian samples. Unlike the other analyses, however, affinities between these Central Asian samples are markedly diffuse. The Central Asian samples link to all other samples via long and very distant affinities with the latest (Sarai Khola: SKH) and earliest (Neolithic Mehrgarh: NeoMRG) of the Indus Valley samples. Remaining samples fall into three aggregates. The first is found in the center-right of the array and included the three living (MDA, MRT, MHR) and one prehistoric (INM) sample from Maharashtra. These are joined by the two Dravidian-speaking caste samples from Andhra Pradesh (GPD, PNT). The tribal Chenchu join this aggregate as a distant outlier, followed by the post-Harappan sample from Timargarha (TMG) and the early Chalcolithic sample from Mehrgarh (ChlMRG). The second aggregate is found in the upper right and includes the highland Pakistani samples of Swatis, the inhabitants of Madaklasht (MDK), Awans (AWAm1, AWAm2), Khowars (KHO), and Wakhis (WAKs, WAKg). As in other analyses, Swatis and the Madaklasht share closest affinities to one another, the two Wakhi samples show closest affinities to one another, as do the two samples Awans, albeit in the latter case to a lesser extent. However, in contrast to the results obtained from multidimensional scaling, it is the second sample of Awans (AWAm2) that serve to link these samples to prehistoric inhabitants of the Indus Valley (HAR). The third aggregate is found in the extreme upper right of the array. This

aggregate includes Tanolis, Syeds, Gujars and Karlals. In this case, Karlals are the most divergent from all other samples, followed by Gujars. By contrast, of the members of this aggregate, Tanolis are most proximate, sharing rather distant affinities to Khowars. Syeds occupy an intermediate position between Tanolis on the one hand and Gujars on the other.

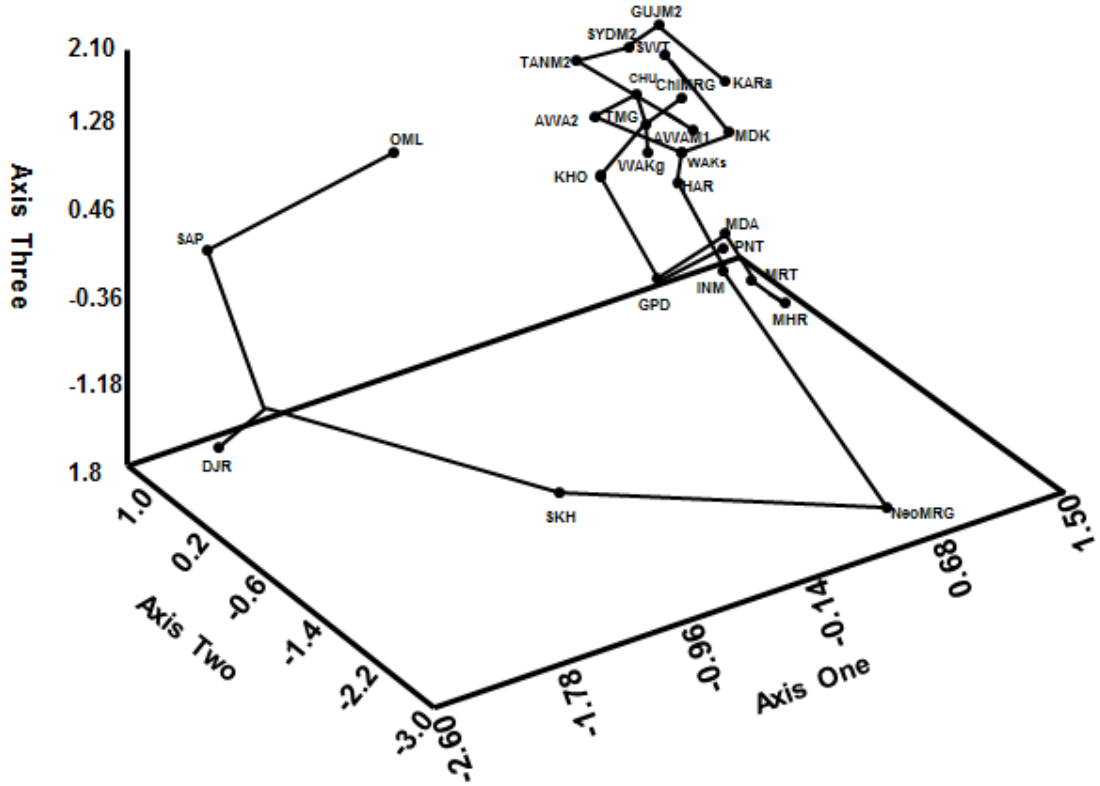


Fig.20. Principal coordinates analysis of all the living and ancient groups of the region

3.2 Mitochondrial DNA analysis

The saliva samples collected from individuals of seven ethnic groups of Districts Mansehra and Abbottabad in accordance with our modified procedure (as described in 2.4.2) were very effective as it precisely yielded high quality genomic DNA of the human buccal cells (Fig. 21).

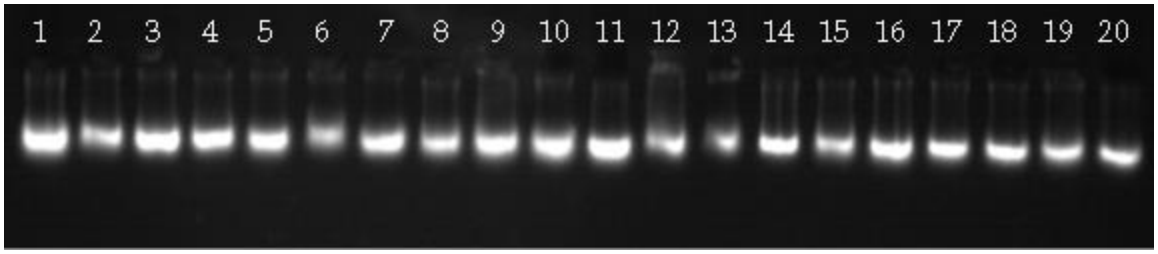


Fig.21. Agarose gel electrophoresis photograph of isolated DNA

Similarly, the amplification of HVS regions of mitochondrial DNA by PCR and its products separation by electrophoresis 1.6% on agarose gel was very successful. The isolation of allele as visible in figure 22 shows the actual band of *mt*DNA HVSI region (451bp long).

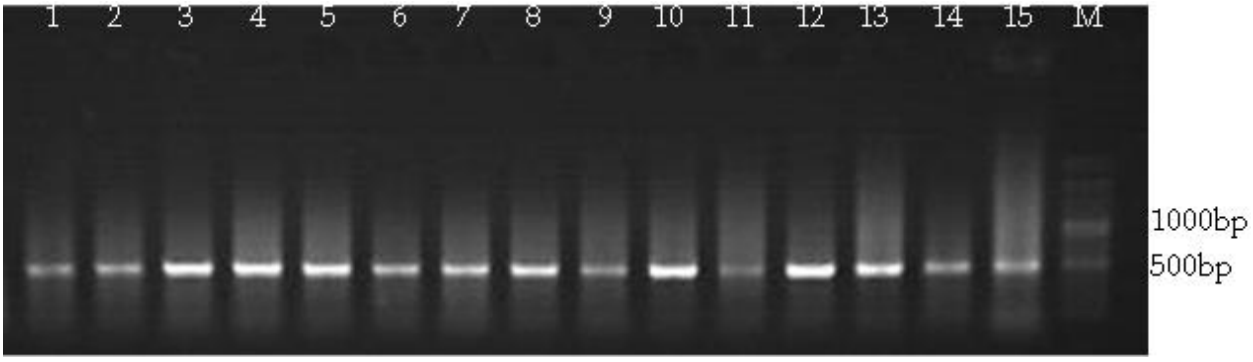


Fig.22. Agarose gel electrophoresis photograph of amplified *mt*DNA HVSI region

Similarly, the successful amplification of the other specific band of *mt*DNA HVSII region, 563bp long after PCR amplification is shown in the figure 23. All these results confirm that our modified procedures for isolation and processing of DNA were very effective and time saving.

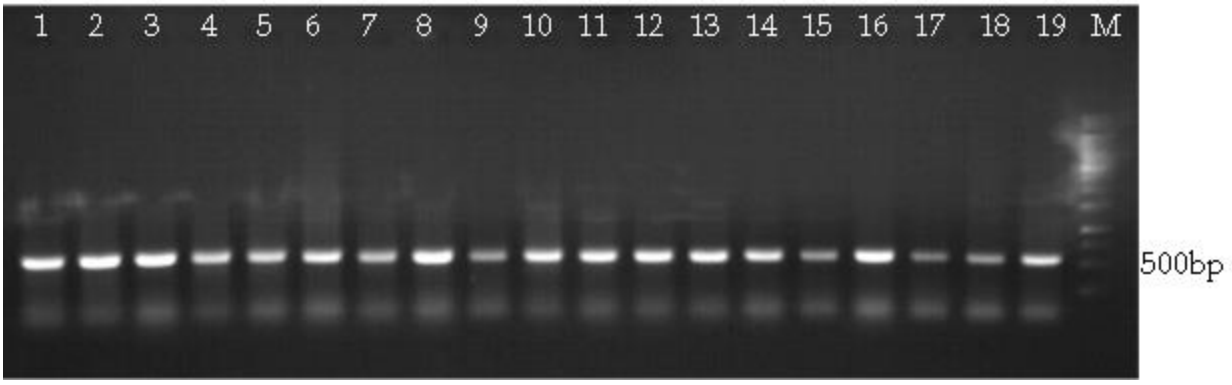
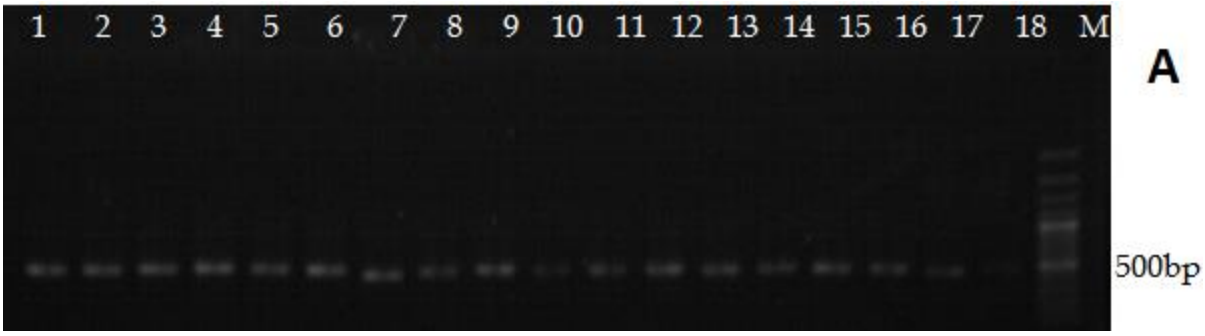


Fig.23. Agarose gel electrophoresis photograph of amplified *mt*DNA HVSII region

Similarly, our improved protocols for elution of the specific DNA fragments (mentioned in 2.8) corresponding to the HVSI and HVSII sequences were also yielded very handsome results as shown in the figure 24.



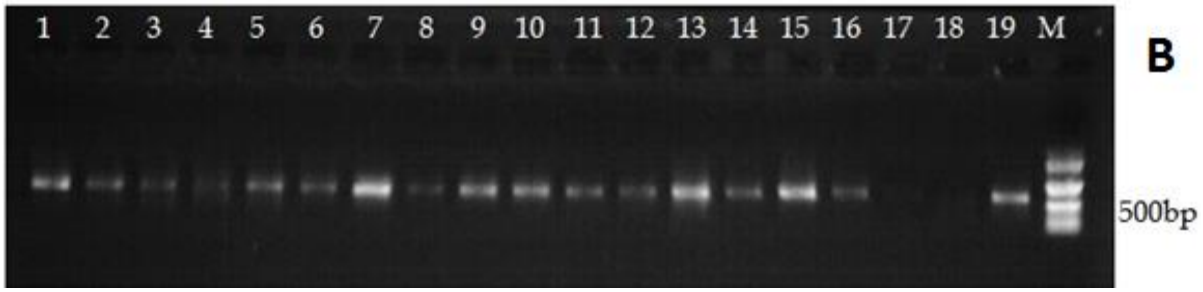


Fig.24. The Agarose gel electrophoresis pictures A and B representing eluted PCR products of *mt*DNA hyper variable sequences I and II respectively

Furthermore our experience of nucleotide sequencing from the extracted DNA of *mt*DNA was very good with Macrogen Korea. The sequences from each resultant peak of particluar popultaions were converted into the FASTA format and blasted against NCBI and the accurate matched sequences were selected for further analyses. The nucleotide substitution, insertion and deletion sites thus identified were transformed into relative frequencies and haplotypes. The analysis was done both for hypervariable sequence I and II in the mitochondrial control region, sequences from 225 (for HVI) and 298 (for HVII).

3.2.1 The HVSI Analyses

Analyses of the HVSI nucleotide sequences revealed 83 haplotypes (Table 7). Among the 83 haplogroups, 39 (47%) were scored once, 15 (18%) twice and 12 (14.5%) three times. More details of the distributions are given in Annexure I.

Table 7: Haplotypes frequencies obtained through *mt*DNA HVSI region analysis

Haplotype	Count	%age
N	14	6.36
H2a2a1c	13	5.91
U7	9	4.09
M4	8	3.64
M3	8	3.64
H	7	3.18
H2	7	3.18
H13b	7	3.18
H5	6	2.73
H6a1a2a	6	2.73
HV2	5	2.27
H1	5	2.27
L3'4	4	1.82
H1ar	4	1.82
X	4	1.82
H2a2b	4	1.82
R5a2	4	1.82
T2b2	3	1.36
H6	3	1.36
W6	3	1.36
M33c	3	1.36
G1b	3	1.36
P6	3	1.36
N1a3	3	1.36
M5a2a1a	3	1.36
M30c1	3	1.36
T2b2b	3	1.36
M3c2	3	1.36
S1	3	1.36
R7	2	0.91
U7a4	2	0.91
H3x	2	0.91
F1b1a	2	0.91
H6b	2	0.91
T1	2	0.91
M33a2	2	0.91
N9a4b	2	0.91
H2a1	2	0.91

I1	2	0.91
D6	2	0.91
M73'79	2	0.91
M49	2	0.91
E2	2	0.91
H1bt	2	0.91
D4m2	1	0.45
H2a3	1	0.45
H2a2b1a1	1	0.45
H11a2a	1	0.45
H24	1	0.45
H3a	1	0.45
R6a	1	0.45
H3b1	1	0.45
V16	1	0.45
J1c2b	1	0.45
C4a2'3'4	1	0.45
U4a1	1	0.45
X2c	1	0.45
J1b5b	1	0.45
H5a1g1	1	0.45
T2b	1	0.45
R0a	1	0.45
M5a2a2	1	0.45
D4b1b1a	1	0.45
H1c3b	1	0.45
D	1	0.45
H1ab1	1	0.45
H1b1	1	0.45
X2e2b	1	0.45
A2	1	0.45
L3a	1	0.45
M5b2	1	0.45
H5e	1	0.45
J1b1a2	1	0.45
D4e5b	1	0.45
H3p	1	0.45
N11a1	1	0.45
J1b	1	0.45
H8	1	0.45

L4	1	0.45
M18	1	0.45
L3d3a	1	0.45
H1aj1	1	0.45
H2a2a1d	1	0.45

The results revealed that the most frequent haplogroup in people belonging to Districts Abbottabad and Mansehra was halogroup H (Table 8), which contained the largest number of individuals scoring 90/225 (40%). The haplogroup M was the next most frequent (Figure 25 & 26), containing 49/225 individuals (21.78%) which are prominent in South Asians (Kivisild *et al*., 2004; Rajkumar *et al*., 2005).

Table 8: Haplogroups distribution in the analyzed people belonging to Mansehra-Abbottabad Districts of Hazara

S.No	HAPLOGROUPS	COUNT	PERCENTAGE
1.	HV	6	2.67
2.	H	90	40.00
3.	L	7	3.11
4.	M	49	21.78
5.	N	35	15.56
6.	R	38	16.89
	Total	225	100

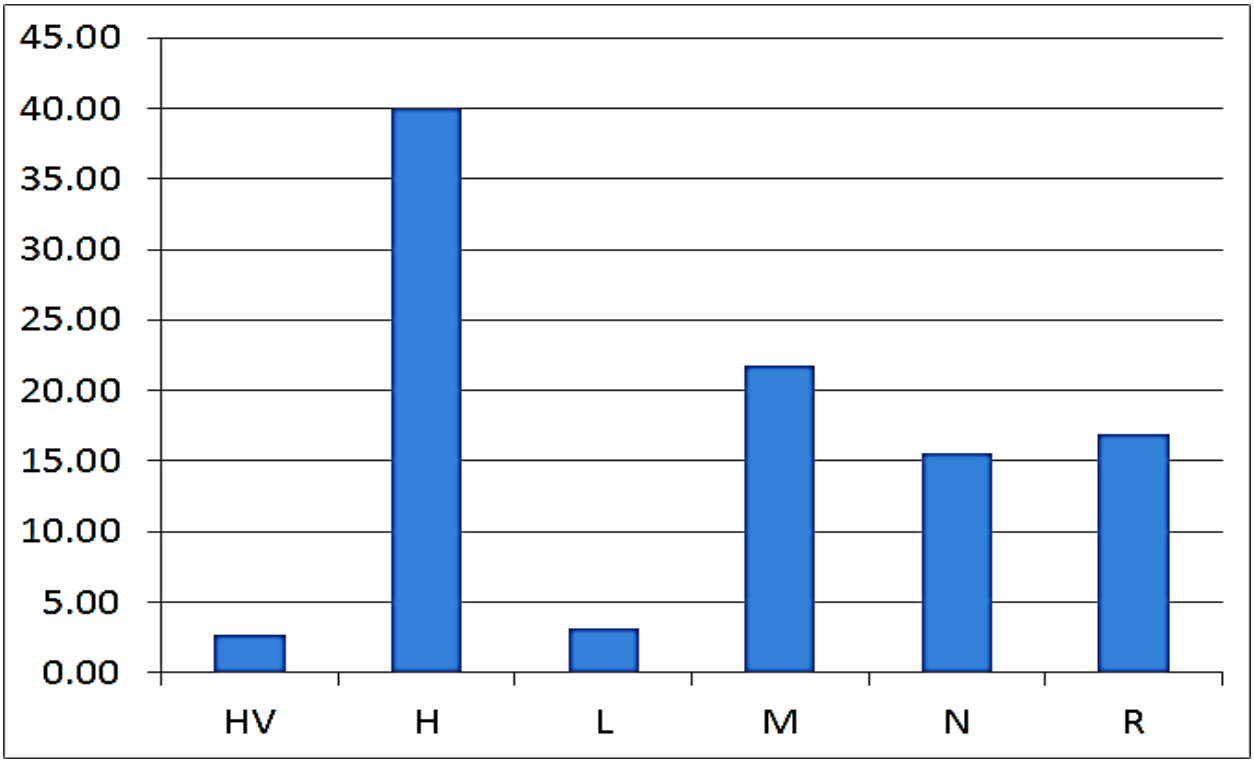


Fig.25. The megahaplogroups recorded in seven tribes of Mansehra and Abbottabad Districts of Hazara

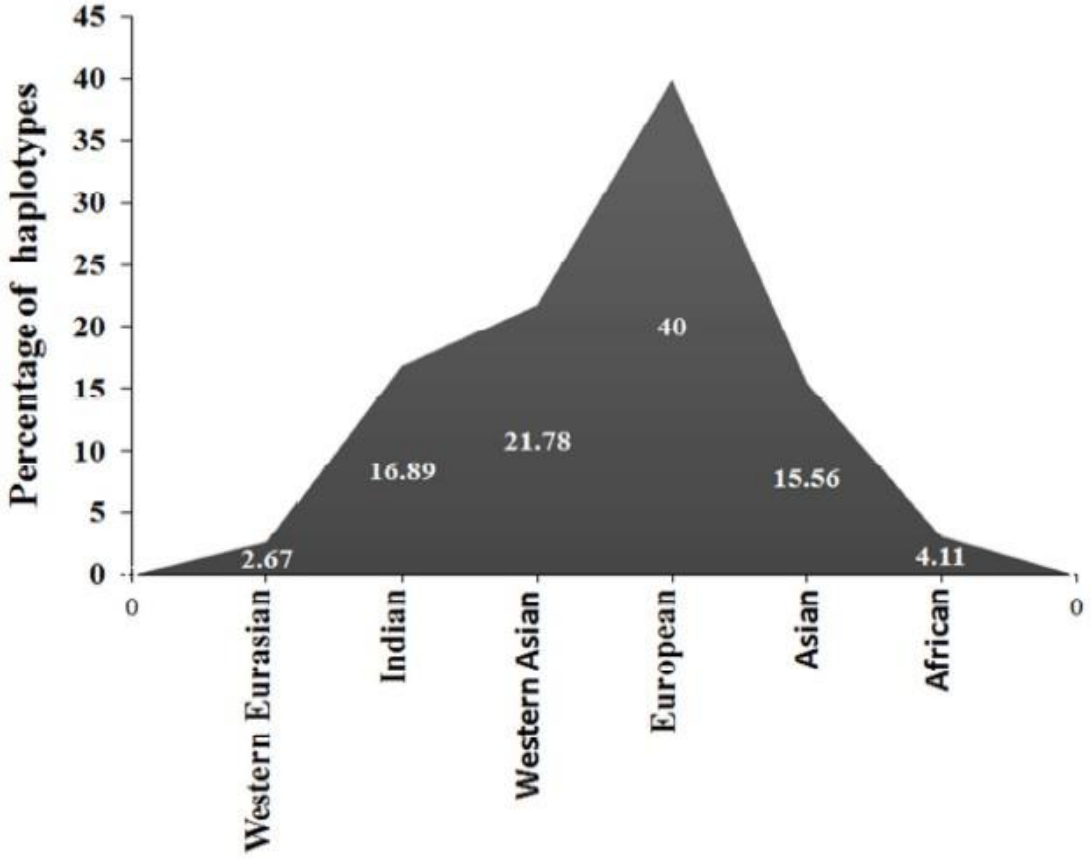


Fig.26. Percentage influence of different populations on the mitochondrial DNA of tribes of Hazara division. Mega-haplogroups observed in seven tribes of Hazara indicate haplotype H (European) 40%, M (Western Asian) 21.78%, R (Indian) 16.89%, N (Asian) 15.56%, L (African) 3.11% and HV (Western Eurasian) 2.67%

The frequency of observed haplogroups in seven tribes of Mansehra and Abbottabad Districts were compared with reported haplogroups in Pakistani populations (Table 9). The highly matched haplogroup is megahaplogroup N. The haplogroups H, HV and M are matched too to a greater extent. Megahaplogroup R found in ethnic groups of the study area match the people with Pathan and Pakistani populations. The other haplogroups matched very little. The haplogroup T was only recorded in Awan population.

Table 9: Frequency of observed haplogroups in seven tribes living in districts Mansehra and Abbottabad, comparing with reported haplogroups in Pakistani populations

Population	N	H*	HV	R	J	T1	T2	U4	U7	N1	X	M	L	Other	Study
Baluch	39	20.50	10.30	---	7.70	---	---	2.60	2.60	5.20	---	33.30	2.60	7.70	Ottoni et al. (2011)
Hazara	23	13.00	4.30	---	---	---	---	8.70	4.30	---	---	30.30	---	26.10	Ottoni et al. (2011)
Pakistani	100	12.00	4.00	2.00	1.00	---	1.00	---	5.00	3.00	1.00	49.00	1.00	8.00	Ottoni et al. (2011)
Pathan	44	4.50	2.30	6.80	6.80	4.50	4.50	---	---	4.60	---	29.50	---	9.10	Ottoni et al. (2011)
Sindhi	23	8.70	4.30	---	---	---	---	---	8.70	8.70	---	30.40	---	---	Ottoni et al. (2011)
Abbasi	27	29.63	---	11.11	---	---	11.11	---	3.70	7.41	---	22.22	3.70	11.11	Present Study
Awan	44	22.73	2.27	6.82	2.27	4.55	4.55	2.27	9.09	13.64	2.27	15.91	2.27	11.36	Present Study
Gujjar	39	58.97	---	5.13	2.56	---	2.56	---	2.56	7.69	2.56	7.69	---	10.26	Present Study
Tanoli	40	35.00	5.00	2.50	2.50	---	2.50	---	5.00	7.50	5.00	22.50	7.50	5.00	Present Study
Syed	33	48.48	6.06	---	3.03	---	---	---	9.09	3.03	---	21.21	3.03	6.06	Present Study
Jadoon	16	56.25	---	6.25	---	---	---	---	---	---	---	6.25	---	31.25	Present Study
Karlal	24	25.00	---	---	---	---	---	---	---	20.83	8.33	16.67	4.17	25.00	Present Study

The quasi-median (QM) network provides an opportunity to examine the quality of *mt*DNA data by graphically representing the genetic structure of the lineages in a data set as shown in Figure 27.

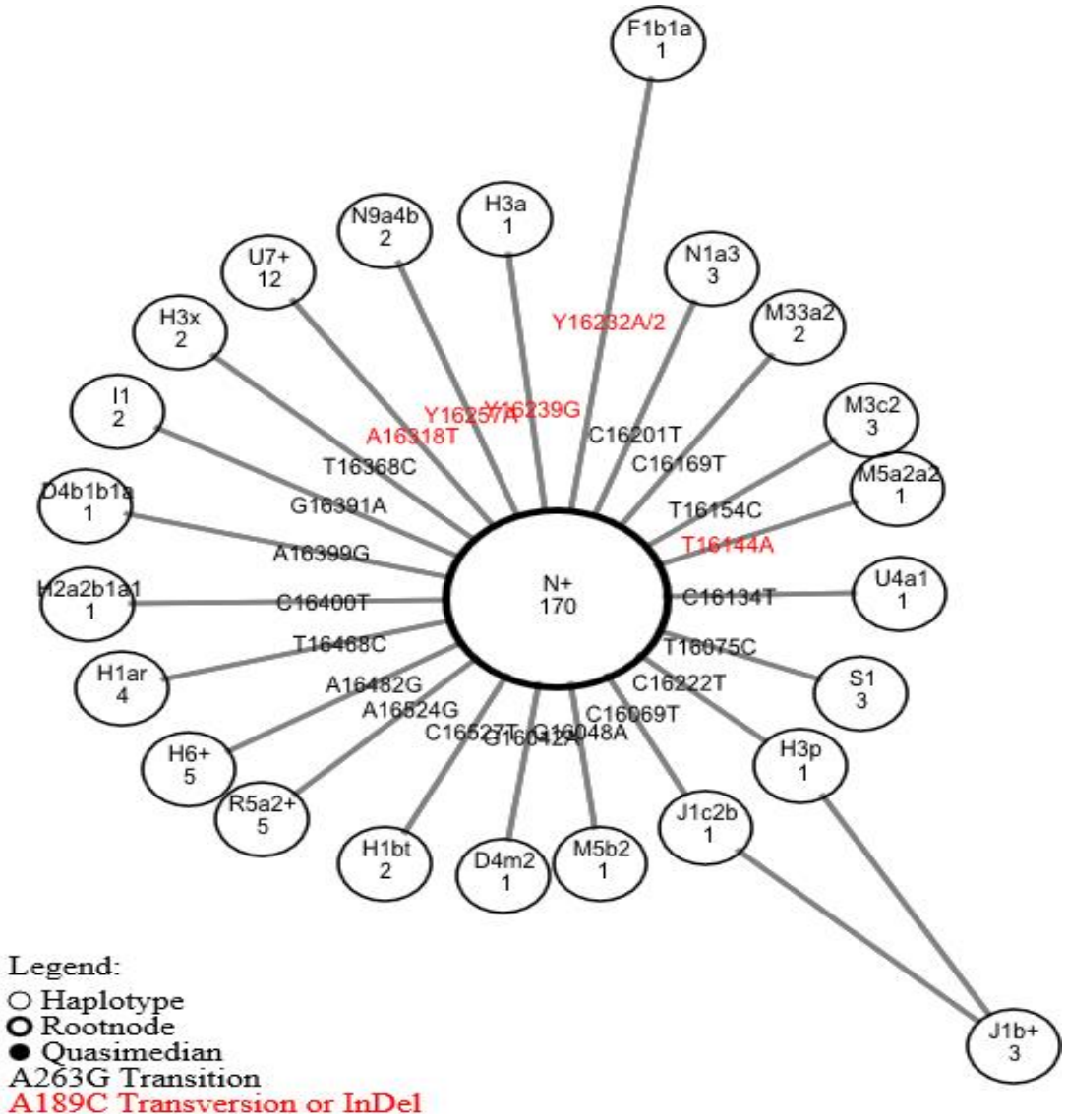


Fig.27. Phylogenetic tree of the mtDNA haplotypes observed in individuals of Mansehra and Abbottabad districts. Haplotypes are shown with circles, with the number of carriers indicated

3.2.2 The HVSII Analyses

The HVSII nucleotide sequences analyses revealed 78 haplotypes (Table 10). Among the 78 haplogroups, 30 (38%) were scored once, 18 (23%) twice and 09 (11.5%) three times. More details of the distributions are given in Annexure II.

Table 10: Haplotypes frequencies obtained through *mt*DNA HVSII region analysis

Haplogroup	**Frequency**	**%**
M	4	1.3
X2d1	4	1.3
Z3a	1	0.3
F1d	1	0.3
M13'46'61	7	2.3
M14	1	0.3
M18a	1	0.3
M2a1a	2	0.7
M3	13	4.4
M30	11	3.7
M30a	3	1.0
M30b	10	3.4
M30c	3	1.0
M33a2a	1	0.3
M34	14	4.7
M35	2	0.7
M37	2	0.7
M39b1	5	1.7
M3c	7	2.3
M44	2	0.7
M65	7	2.3
M70	2	0.7
M71a1	1	0.3
A4	3	1.0
I1	3	1.0
N1a	1	0.3
N1a1'2	2	0.7
N1a3	3	1.0
N2	2	0.7
W	8	2.7

W3b	2	0.7
W4	2	0.7
X1'3	1	0.3
X2d	11	3.7
H	2	0.7
H15	1	0.3
H15a1b	1	0.3
H1an2	2	0.7
H1av1	1	0.3
H1c	1	0.3
H2a2a2	8	2.7
H2a2a2	10	3.4
H3	9	3.0
H32	52	17.4
H57	1	0.3
H5a3	2	0.7
H6	3	1.0
HV0	1	0.3
HV0f	1	0.3
HV2	6	2.0
HV2a	1	0.3
J	1	0.3
J1b1b	1	0.3
J1c16	1	0.3
J1c3i	2	0.7
J1d	1	0.3
J2a2	1	0.3
K1e	3	1.0
K2a5	1	0.3
K2a8	2	0.7
R0a4	1	0.3
R11	2	0.7
R2	2	0.7
R24	2	0.7
R30b	4	1.3
R30b1	3	1.0
R5a1	1	0.3
R6a1	3	1.0
T2d1b	1	0.3

U1a1	1	0.3
U2b2	8	2.7
U2e1	6	2.0
U2e1'2'3	2	0.7
U3	1	0.3
U4'9	1	0.3
U4a1	1	0.3
U4a2	1	0.3
U4a3	1	0.3

The results revealed that the most frequent haplogroup in people of Districts Abbottabad and Mansehra was halogroup R (Table 11), which contained the largest number of individuals scoring 160/298 (53%). The haplogroup M was the next most frequent (Figure 28), containing 97/298 individuals (33%) which are prominent in South Asians (Kivisild *et al*., 2004; Hofmann *et al*., 1997 and Macaulay *et al*., 1999).

Table 11: Haplogroups recorded in Mansehra-Abbottabad Districts through *mt*DNA HVSII analysis

S.No	**HAPLOGROUPS**	**COUNT**	**PERCENTAGE**
1.	N	40	13.4
2.	M	99	33.3
3.	R	159	53.3
	Total	298	100

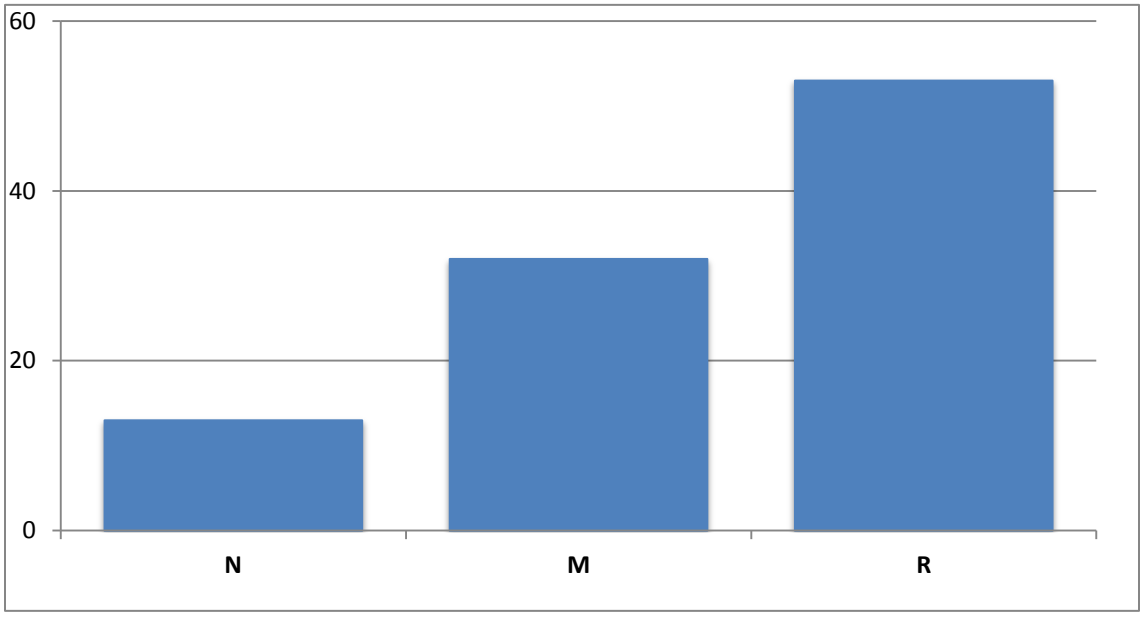


Fig.28. Megahaplogroups recorded in seven tribes of Mansehra and Abbottabad Districts through *mt*DNA HVSII analysis

The quasi-median (QM) network for *mt*DNA HVSII examine the quality of *mt*DNA data by graphically representing the genetic structure of the lineages in a data set as shown in Figure 29.

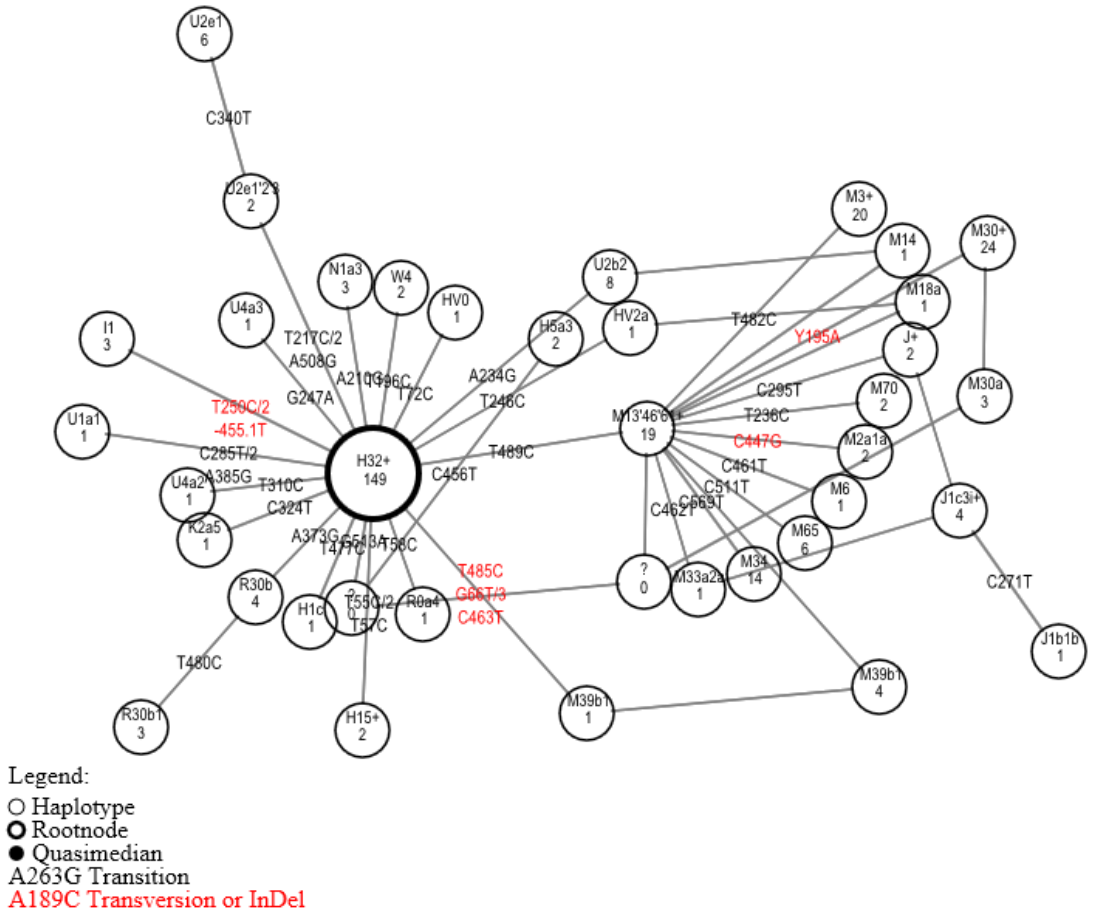


Fig.29. Phylogenetic tree of the *mt*DNA HVSII haplotypes observed in individuals of Mansehra and Abbottabad Districts. Haplotypes are shown with circles, with the number of carriers indicated.

DISCUSSION

South Asia, constituting mainly the Indo-Pak sub-continent, is a panorama of social diversities, racial differences and rich cultural heritage (Bhasin, 2006). Many facets of history, race and culture of this area are still unexplored. Debates and doubts centering on the autochthonous status of the early settlers continue. It is very difficult to ascertain how human groups and settlements were formed in the pre-historic times, whether they were the original inhabitants or migrated from some other place and if they migrated, which route did they take. However, the infiltration and admixture of new racial and cultural elements, from time to time (Lukacs and Hemphill, 1991), have made the Indian population more diverse and heterogeneous. An extensive account on the history of the area can be reviewed in Sankalia (1962, 1979) and Allchin (1983). From the fourth century B.C. onwards for 2000 years, the areas included in Pakistan were subjected to repeated waves of penetration by alien peoples. Greeks, Parthians, Scythians and Pahlavas including the Kushans were the first to come in after the Indo-Aryan civilization entered its settled course (Maloney, 1974; Thapar, 1969). The Huns came in somewhat larger numbers at the close of the Gupta epoch. These successions of peoples from outside India were assimilated into Hindu society. On the west coast, Jews and Parsis came after fleeing from their own homelands. Arabian Muslims, Persian Muslims, Turks and Afghans, whose total number was very large came to India at time to time. The Muslim immigration into India began even before the Arab invasions of the Sind quite early in the eighth century A.D. and ended with the establishment of the Mughal Empire in the sixteenth century. This was the last major movement which

produced some perceptible changes in the composition and culture of the indigenous population. The Muslims did not accept the Hindu religion but they made converts to their religion.

Mourant (1983) is of the opinion that it is almost certain that man evolved from his pre-human ancestors and emerged in tropical Africa however the Indo-Pak apparently derived much of its Neolithic as well as its Metal Age Civilization from two sources i.e. one along the Makran Coast and Baluchistan and the other from southeast Asia by way of the Arakan coast and Assam (Fairservis, 1971; Bhasin, 2006). The knowledge of the first source is documented from numerous sites in the arid regions along the foothill rim of the Indus valley, the second is still largely covered with dense vegetation and is only beginning to be understood (Bowles, 1977). Using five polymorphic restriction sites on b gene cluster Long *et al*. (1990) worked out the evolutionary history and affinities among Africans, Eskimos and Pacific Island populations, then reported an African origin of the modern *Homo sapiens*. It is believed that man entered Asia from Africa, probably over one million years ago; bones of the early human species, *Homo erectus* have been found in China and Javaas well, which could have reached only through Asia. Moreover, to reach Europe, which he probably very soon did, he must have passed through south-west Asia. A review of the literature shows that Pakistan, as a connecting link between east and west has played a major role for the dispersal of modern humans out of Africa (Cann, 2001; Underhill *et al*., 2001; Cavalli- Sforza and Feldman, 2003; Kivisild *et al*., 2003; Palanichamy *et al*., 2004 and Cavalli- Sforza, 2005). It

was probably in Asia that Eurasian man, by now of the modern Homo sapiens species, diverged from African man, and then became differentiated into Caucasoid and Mongoloid types. Another differentiation, which probably took place in Asia, is that of the Australoids, perhaps from a common type before the separation of the Mongoloids. The Caucasiods and the Mongoloids almost certainly became differentiated from one another somewhere in Asia and Caucasoids subsequently spread to the whole western part of the continent and thence to Europe and North Africa (Mourant, 1983). The divergence lines among the three major races; Negroid, Caucasoid and Mongoloid estimated by Nei and Roychoudhury (1984).

Biological/physical anthropology studies inter and intra-population variations, dental morphology and *mt*DNA are some of the important areas of genetic analyses used for elaborating phylogenetic relationship among and in between human kinds. It can be noted that physical anthropology is a descriptive study of biological parameters or the use of mathematical models for understanding the causes of variation and co-existence of genetic traits. Pakistan is a multicultural country (Bellew, 1880 and Rose, 1911), its populations offer a great opportunity of genetic diversity which varies from African to the Mongoloid and nearly all the intermediates available elsewhere in the world. The study area which includes two districts of Hazara Division viz. Abbottabad and Mansehra, where people of different groups diverse in ethnicity, castes and tribes live in a small geographic area entirely act symbiotically towards the natural resource, are

living side by side for hundreds or even thousands of years have generally retained their separate entities by practicing endogamy.

Little attention has been paid to the biological evidence of skeletal records and genetic distances (Kennedy, 2000), as available in the nucleotide sequences and mutations in genes occurred from time to time. As such the despite evidence of population movements and shifts in the inter-regional context, the temporal depth and patterning of biological interactions remain largely uninvestigated. Genetic data on the Pakistani populations though scanty has shown differences between the Pakistani and other world populations. Most of the earlier studies included Pakistani populations as a single entity which is incorrect because Pakistan consists of more than 12 different ethnic groups (Wilson-Wilde *et al.,* 1997; Forman and Lambert, *2000* & Zhivotovsky *et al.,* 2001). The few studies that are available with reference to Pakistani ethnic groups have shown clear differences among them (Mansoor *et al.,* 1998; Mehdi *et al.,* 1999 and Qamar *et al.,* 1999). On the basis of these studies certain populations present in the northern and southern parts of the country can be distinguished.

The population history of the numerous ethnic groups of the Indo-Pak subcontinent has long been the subject of inquiry (Basu *et al*., 2003; Barnabas *et al*., 2006; Chaubey *et al*., 2007 and Kivisild *et al*., 2003). Current views may be organized into three competing models. The first may be termed as the *Long-Standing Continuity Model* (Hemphill, 2013), which trace back the foundations of the modern population of South Asia to the initial dispersal of *Homo sapiens* out of Africa some 62-75 thousands years ago. The proponents

of this model are of the opinion that the South Asian populations, once established, have been little affected by gene flow from populations adjacent to the subcontinent or by any substantial population movements within the subcontinent (Sahoo *et al*., 2006; Krithika *et al*., 2009; Kennedy *et al*., 1984; Epperson, 1993 and Manel *et al*., 1993). Consequently, the pattern of biological affinities among living ethnic groups of South Asia is held to be a function of simple isolation-by-distance such that populations most similar in antiquity and in geographic proximity are most similar biologically.

The second model generally referred to as *Aryan Invasion Model* (Hemphill, 2013), stress on a series of innovations that occurred among the Bronze Age populations of Central Asia, such as domestication of the horse and the development of the war chariot (Bryant, 2001; Renfrew, 1987; 1996 and Jarrige, 1984). They note the presence of Indo-Aryan languages throughout the northern two-thirds of the subcontinent, and descriptions of horse mounted invaders conquering the citadels of the noseless *Dasus* in the *Rg Veda* as evidence of a Central Asian invasion into the subcontinent during the mid of 2nd millennium BC.

The third model may be termed as the *Out of India Model*. Proponents of this model are of the opinion of early appearance of agriculture and complex cities, both within the Indus Valley and the Doab of North India (McAlpin, 1981), suggest that South Asian populations dispersed outward into adjacent regions of southwestern and Central Asia. However, proponents of this model disagree over the timing of this dispersal. In one version, South Asia is held to be the homeland of Indo-European languages, from

which populations speaking these languages dispersed during the early 3rd millennium BC. In the second version, this dispersal is claimed to be far later, during the early Iron Age of the 1st millennium BC.

Dental variation, the heritable dental traits are caused by multiple genes and are little influenced by environmental factors (Scott and turner, 1997). Dental morphology in the anthropological sense, involves observations of the minor variations in the cusps, ridges, grooves and root structures, can be used for differentiating populations (Hillson, 1996; Dahlberg, 1945; Pedersen, 1949 and Moorrees, 1957). The observation, that dental traits exhibit significant differences in frequency among major geographic areas (Dahlberg, 1945; Dahlberg, 1951 and Hrdlicka, 1920), and in some cases traits, the differences among groups are so obvious that the Mongoloid, Caucasoid and African dental complexes are easily differentiable (Buikstra *et al.,* 1990; Haeussler, 1989; Hanihara, 1968; Irish, 1994 and Mayhall *et al.,* 1982).

The present research provides the first hand information to examine biological affinities of the samples based on dental morphology. The research further offers the opportunity to test if and when there were major introductions of foreign genes into the resident South Asian gene pool and whether the samples in the study are associated with any of these alleged population movements. Lastly, it is also the first effort to analyze dental anthropological data from living ethnic groups of northern Pakistan and to test whether any biological link exists between the living inhabitants of the Mansehra and Abbottabad Districts of KP Pakistan and the ancient inhabitants of the Indus Valley or elsewhere within the Indian Subcontinent.

Pairwise differences among samples were assessed with Smith's MMD and these pairwise distances were submitted to four different data reduction techniques. These data reduction techniques include: hierarchical cluster analysis, neighbor-joining cluster analysis, multidimensional scaling and principal coordinates analysis.

The results revealed that if the Long-Standing Continuity Model is presumably true i.e. human populations in South Asia established some 75,000 years ago and that there has been no substantial gene flow from adjacent populations or significant migrations within the subcontinent, the patterning of the biological affinities available among the ethnic groups can be marked by temporal and geographic propinquity. This ought to be reflected by regional structuring of peninsular Indians, Central Asians, Indus Valley inhabitants, Hindu Kush, Himalayan highlanders and inhabitants of the northern Indus Valley Periphery; as well as by temporal structuring between prehistoric Central Asians, prehistoric inhabitants of the Indus Valley and all living people. On the other hand, if the Aryan Invasion Model is correct, that innovations among Bronze Age Central Asians led to an invasion of South Asia during the mid of second millennium BC, then biological distances ought to reflect a discontinuity in Indus Valley biological history that coincides with the dissolution of the Harappan Civilization such that all post-Harappan samples are the descendants of these Central Asian Aryan invaders. Furthermore, if it is true that this invasion ushered in speaking the Indo-European languages who subsequently spread to the Doab of North India, then biological distances ought to reflect the close affinities between prehistoric Central Asians and

peoples from North India. However the biodistances of Dravidian speaking people from southeast India ought to reflect strong biological segregation from these alleged invaders and their North Indian descendants. Finally, ethnic groups of the Hindu Kush and Himalaya highlands including the people of Hazara and the northern periphery of the Indus Valley ought to exhibit affinities to these Central Asian invaders. Our results as provided in the result section shows that the people of Abbottabad and Mansehra whatsoever their way or more of assembling into the area was; belong to the same origin i.e. from Central Asian invasions and their northern Pakistani descendants. The existence of variations in between these groups is apparently geographic due to isolation, appearance/arrival of the people in Hazara, and social-cultural isolation in the past. If, however, the first version of the Out of India Model is correct, that the rise of complex cities in the Indus Valley signals the genesis of population dispersal to adjacent regions of southwest and Central Asia during the 3rd millennium, then the biological history of South Asian populations ought to be marked by long term isolation with considerable regional and temporal structuring, coupled with a narrowing of biological distances between late Bronze Age Central Asians and post-Chalcolithic populations of the Indus Valley, North India, and northern Pakistan. However, if the second version of this model is true, that the rise of complex cities that sparked this dispersal out-of-India is signaled by urbanization in the Doab of North India, then dispersal of South Asian populations into adjacent regions of southwest and Central Asia did not occur until the mid of first millennium BC. Consequently, prehistoric Central Asian samples from the late Bronze Age, since they antedate the dispersal,

should exhibit no settlement with any of the South Asian samples included in this analysis, for this did not occur until the Iron Age.

Examination of the biodiversity among and in between the living ethnic groups of Northern Pakistan suggests a fundamental split between Karlals, Gujars and Syeds. Among the remaining ethnic groups, affinities are consistently close between the two geographically distinct samples of Wakhis, which confirms Wakhis at least has retained the self-identifying ethnic identities and can easily be taken as a standard for identification and differentiation. In case for Awans all the analyses indicate that the phenetic affinities between the samples collected even from different villages of Mansehra District are not that clear which can be taken as standard for the group. The samples of Khowars are interposed in between the Awans as visible in figure 11. Neighbor-joining cluster analysis and MDS with Kruskal's method indicate that sample of Awans (AWAm2) is much more proximate phenetically to Khowars, than the sample of Awans collected by Hemphill (AWAm1). The Principle Coordinate analysis shows that the sample of Awans collected by Hemphill (AWAm1) is identified as possessing closer affinities to Khowar than the samples of Awans (AWAm2) collected and analyzed during the present study. All analyses indicate that the Awans samples link Awans to the Swatis and Madaklasht on the one hand and to the two Wakhi samples on the other. Analysis through MDS and PCO indicates that the affinities between AWAm1 and Swatis are much closer than the affinity between the sample of Awans and the inhabitants of Madaklasht. All analyses, except PCO indicate that the samples

of Wakhis from Gulmit (WAKg) are more similar phenetically to Hemphill's sample of Awans than is the sample of Wakhis from Sost.

Analysis of the biological affinities of major tribes residing in Northern Pakistani, in context of the living ethnic groups from peninsular India and prehistoric dental samples collected from Indus Valley and South-central Asia have yielded several consistent anthropological patterns i.e. the prehistoric South Central Asians are clearly separated from both living and prehistoric South Asian samples (Fig. 16) and the living ethnic groups of Northern Pakistan show closest affinities to one another; with an exception i.e. the two geographically distinct samples of Wakhis, which are very similar to one another (Fig. 16). The two samples of Awans do not show close affinities to one another, instead, Khowars from Chitral District interposed between the Awans from different villages. Moreover the Awans of Mansehra is marked by secondary affinities to the two Wakhi samples, and more distantly to the Madaklasht and Swatis from Mansehra and shares secondary affinities with Tanolis of Mansehra, which link, though distantly with Syeds, Gujars and Karlals. The results analyzed by Neighbor-joining cluster analysis revealed substantial regional structuring of South Asian samples i.e. Southeast peninsular Indians occur in the left-center, west-central Indians the right-center, while the Pakistani ethnic groups of the northern periphery of the Indus Valley occupy the lower center-right (Fig. 17). These latter ethnic groups are split between Awans, Tanolis and Swatis, who share affinities with Hindu Kush highlanders, and those, such as Syeds, Karlals, and Gujars who shows distinct and closed phylogenetic affinities.

Central Asians are segregated on the left side, while prehistoric Indus Valley samples are widely dispersed. Analysis of the data through multidimensional scaling revealed (Fig. 18 & 19) that all the living ethnic groups from south-east and west-central India occupy the right front of the array. While people from northern Pakistan occupy the upper-back right and lower central position, which splits the ethnic groups between Awans, Tanolis and Swatis in the upper right with affinities, while Syeds, Gujars and Karlals to lower center; Central Asians occupied the left side, while prehistoric Indus Valley samples occupied the forward center. These samples are widely dispersed and show no temporal or geographic structure. The same patterns occur within the principal coordinates analysis. Prehistoric Central Asians are segregated on the left side of the array. Southeast and west-central peninsular Indians occur in the lower right, while Pakistani people occupied the upper left. The absence of temporal and geographic structuring of the Indus Valley samples is once again evident.

A synthesis from our results analyzed with the available information shows that the ethnic groups of the northern Pakistan, based on the patterns of their phenetic affinities can be divided into two main groups among which, the Awans, Tanolis and Swatis are affiliated with ethnic groups of the Hindu Kush Himalayan highlands, while Syeds, Karlals and Gujars segregates uniquely and possess no affinities to any of these groups. The reasonable possibility of the close affinities of Syeds, Karlals and Gujars might be their origin from the same stock in the near past as compared to Awans, Tanolis and Swatis, who inhabit the same area for almost 300 years back. Their aggregations in the

same geographic proximity have not distributed their individual gene pools due to their tribal nature and no cross marriages. The distances among Awan population of the nearly collection sites in Mansehra and their close affinities with the people of far placed Wakhis and Khowars needs further explanations. Furthermore the ethnic groups of peninsular India are most similar to one another and aggregate by geographic region. The prehistoric people of Indus Valley showed no temporal or geographic continuity, nor do they share any affinities with living ethnic groups from Mansehra and Abbottabad.

Complete record of the molecular anthropology of the castes and tribes of the Pakistan is unavailable, however the higher haplogroup diversity than the other regions, probably suggesting their native status, and serve as a major passage of modern human scattering out of Africa (Cann, 2001). The availability of variety of population with different morphological, genetic, cultural and ethnic characteristics in Pakistan shows the repeated appearance of invasion here. A number of *mt*DNA studies that focused on the hypervariable segments (HVSI and HVSII) applied to various Indian populations and have provided some insights into the genetic structure of the populations of the area (Kaur *et al*., 2002; Basu *et al*., 2003; Kivisild *et al*., 2003*a*; Roy *et al*., 2003; Hofmann *et al*., 1997 and Macaulay *et al*., 1999). Analysis of the total phylogeny of all *mt*DNA lineages of India and Pakistan is partially linked with the Western Eurasian *mt*DNA phylogeny but includes abundant basal branches that are absolutely absent in Europe (Kivisild *et al*., 1999*a* and 1999*b).* The ancestral population probably entered Pakistan

either from the west via Iran or the north via Central Asia (Quintana-Murci *et al*., 2004). The gene flow was apparently more limited in the opposite direction and not very far-reaching: for example, Quintana-Murci *et al*. (2004) reported 4 haplogroups i.e. one each R5 and N5, and 2 M lineages out of 42 *mt*DNA lineages from central Iran but only one distinct lineage from macrohaplogroup M out of 95 in Northern and Western Iran that potentially belong to the South Asian haplogroups. Only one R5 lineage was reported in Iraqi samples (Al-Zahery *et al*., 2003). Farther to the north-west, in the Caucasus area and Turkey, such lineages are virtually absent. In the Central Asian data set of Comas *et al*., (2004), only 6 out of 232 lineages belong to South Asian haplogroups, among which 2 from U2a, 1 from U2c, 2 from R5, and possibly 1 from M4 whereas the Western Eurasian *mt*DNA lineage assembled into haplogroups HV, N1I, N2W, R1, R2, JT, UK and X (Torroni *et al*., 1998; Macualary *et al*., 1999; Tambets *et al*., 2004).

The lineage analysis of *mt*DNA phylogeny shows that all the *mt*DNA haplogroups outside of Africa are descendants of either haplogroup M or its sibling haplogroup N (Metspalu *et al*., 2004). The geographical distributions of M and N out of Africa migrations and their subsequent habitation in the rest of the world, is often considered as a single major prehistoric migration of humans out of Africa, which shows that M and N haplogroups were part of this habitation process (Macaulay *et al*., 2005). Haplogroup M has also not penetrated west of the Indus Valley, although it is present at high frequencies in south Pakistani and Indian populations. Thus, the distribution and ages of these lineages suggest that they are the legacy of the first inhabitants of the

south-western Asian region who underwent important expansions during the Paleolithic period. The highest frequencies worldwide of haplogroup M is observed in Asia, specifically in Bangladesh, India, Japan, and Tibet, where its frequency ranges from 60%-80%. The total frequency of M subclades is even higher in some populations of Siberia or the Americas, but these small populations tend to exhibit strong genetic drift effects, and often their geographical neighbor's exhibit very different frequencies (Rajkumar *et al*., 2005; Thangaraj *et al.,* 2006). Only two subclades of haplogroup M i.e. M1 and M23, are found in Africa, numerous subclades of M are distributed outside Africa (Rajkumar *et al., 2005*; Gonzalez *et al*., 2007), but according to Gonzalez *et al*. (2007) the M1 appears to have expanded recently. The haplogroup H is a descendant of haplogroup HV. The Cambridge Reference Sequence of the human mitochondrial sequence to which all other sequences are compared, belongs to the haplogroup H2a2a. Several studies conclude that haplogroup H probably evolved in West Asia some 25,000 years ago and was shifted to Europe by migrations between 20-25,000 years ago (Pereira *et al*., 2005; Richards *et al*., 2000). The spread of subclades H1, H3 and the sister haplogroup V reflect a second intra-European expansion from the Franco-Cantabrian region after the last glacial maximum nearly 13,000 years ago (Achilli *et al.,* 2004; Richards *et al.,* 2000). Haplogroup R is a mutant of haplogroup N. Among its descendant haplogroups are B, U (and thus K), F, R0 (and thus HV, H, and V), and the ancestral haplogroup of J and T. In India the castes and tribes of the southern region has higher haplogroup diversity as exhibited in other regions (Maji *et al.,* 2008; Soares *et al.,* 2009).

The polynucleotide sequences obtained in the present study were compared with the human mitochondrial DNA sequence available in the gene bank. We recorded person to person variation in the HVSI and HVSII indicating its importance in the forensic analyses and population genetics. The results reflected a similar impression to that of the previous studies (Miroslava *et al*., 2001; Malhi *et al*., 2003; Bermisheva *et al*., 2004; Barnabas *et al*., 2005; Tamang *et al*., 2012). All the sequence lineages of the seven populations, from Mansehra and Abbottabad, were placed in a set of haplogroups within macrohaplogroups N and R. The haplogroup N had the subclades X, W and N1b whereas R was represented by subclades R0a, H, V, HV, U, K, J and T. The predominant distribution of N and R is a characteristic of West Eurasians, with a representative component from South Asia i.e. M Haplogroup. A very little contribution from Sub-Saharan haplogroup L was recorded here (Olivieri *et al*., 2006; Gonder *et al*., 2007; Abu-Amero *et al*., 2007). The results revealed that the genetic pattern of seven tribes of Mansehra and Abbottabad Districts is similar to the West Eurasia reported earlier (Achilli *et al*., 2004; Pereira *et al*., 2005; Richards *et al*., 2000; Kivisild *et al*., 2004; Rajkumar *et al*., 2005). The HVSI analyses shows that among all the haplogroups the most frequent was H, present in 29.63, 22.73, 58.97, 35, 48.48, 56.25 and 25 percent of Abbassi, Awan, Gujar, Tanoli, Syed, Jadoon and Karlal, respectively. The sister clade T was quite frequent in Abbassis (11.11%) and U7 was the least frequent group observed in 9.09, 5 and 9.09 percent of Awans, Tanolis and Syeds, respectively. Haplgroup N1 was found frequently 20.83% in Karlals. Moreoover, haplogroup M that is prevailing in Pakistan was observed with a frequency of 22.22% in Abbassi, 15.91% in Awans, 7.69% in Gujjar,

22.50% in Tanoli, 21.21% in Syed, 6.25% in Jadoon and 16.67% in Karlal tribes. The HVSII analyses revealed that the most frequent haplogroup was R, present in 18, 13, 15, 14, 8, 15 and 17 percent of Awan, Abbassi, Jadoon, Gujjar, Karlal, Syed and Tanoli, respectively. Halplogroup M was observed with frequency of 13% in Awans, 20% in Abbassis, 20% in Gujars, 13% in Jadoons, 10% in Karlals, 12% in Syeds and 11% in Tanolis.

It has been found that the ancestral node of the phylogenetic tree of all the *mt*DNA types typically found in Central Asia, the Middle East and Europe are also to be found in South Asia at relatively high frequencies. The most frequent *mt*DNA haplogroups in the Indian subcontinent are M, R and U (Quintana-Murci *et al*., 2004; Kivisild *et al*., 1999) which is also confirmed here. Arguing for the longer term rival Y-Chromosome model of Sengupta *et al*. (2006), Oppenheimer believes that it is highly suggestive that India is the origin of the Eurasian *mt*DNA haplogroups which he calls the Eurasian Eves. According to Oppenheimer it is highly probable that maternal lineage of nearly all human is Central Asia; the Middle East and Europe descended from only four *mt*DNA lines that originated in South Asia 50,000-100,000 years ago (Stephen, 2004).

The datasets from all seven populations combined and scrutinized by quasi-median network for identifying unusual polymorphism, showed that *mt*DNA data was also consistent with anthropologic and linguistic theories which suggest that the migration of early Indo-European speaking farmers took place from West Asia into Europe and India (Renfrew, 1992). The high frequency of haplogroups H, R and M observed

confirms the lineage of these populations to Europeans and South Asians. Furthermore variation in the frequency distribution of subclades or macrohaplogroups can be attributed due to the accumulation of particular mutations in the tribes due to geographic or social isolation of the people, or it may also be an attribute of the migration/colonization distance of the people residing in the area.

CONCLUSIONS AND RECOMMENDATIONS

Our results regarding the phenetic affinities among the selected tribes of Abbassi, Awan, Gujar, Jadoon, Karlal, Tanoli and Syed compared with other secondary information available for Northern Pakistani ethnic groups, remains highly consistent throughout the various data reduction techniques. The consistency in phenetic affinities was even evident when they were considered in the backdrop of living ethnic groups of peninsular Indians and prehistoric inhabitants of the Indus Valley and South-Central Asia. When consideration was limited to the people of Northern Pakistan, the uniqueness of Syeds, Gujars and especially Karlals was emphasized. Tanolis served as bridge for linking highly divergent samples to the remaining samples. Among the remaining samples, repeated close affinity was observed between the two geographically distinct people of Wakhis suggested that they belong to the same genetic stock. The samples of Awans and Swatis were divergent from all other ethnic group samples from northern Pakistan, but always show affinities to one another and Madaklashts. A comparison of the people from Abbottabad and Mansehra emphasize the uniqueness of the four prehistoric samples from south-central Asia, which shared no affinities to South Asians, either living or prehistoric. Nevertheless, even when these prehistoric inhabitants of southern Central Asia are included in array of samples considered, the phenetic uniqueness of Syeds, Gujars and Karlals relative to the other ethnic groups of Northern Pakistan remains evident. Standing in contrast to this consistency in phenetic affinities, the degree of affinity between Swatis and the

inhabitants of Madaklasht proved volatile with the different data reduction techniques yielding discordant results.

This research reveals a fundamental lack of regional structure among prehistoric samples from the Indus Valley. This lack of structure stands in contrast to the regional structure observed among the samples of living ethnic groups of northern Pakistan. This contrast in regional structure has direct implications for the population histories of the ethnic groups of Pakistan. If the Aryan Invasion Model is correct, there should be close biological affinities between late prehistoric inhabitants of the Indus Valley, living North Indians, the inhabitants of Hindu Kush and Himalayan highlanders, members of ethnic groups of the northern periphery of the Indus valley and prehistoric Central Asians. After all, the latter are maintained by proponents of the Aryan Invasion Model to be the source population for the former, whose entry into South Asia may be traced to the mid-second millennium BC. This model is not supported, for none of the analyses reported here yield any evidence between South Asian populations, either prehistoric or living, with the prehistoric sample from southern Central Asia.

An alternative interpretation to the Aryan Invasion Model maintains that gene flow between populations of Central Asia and South Asia did, in fact, occur. However, the polarity was reversed; that is, emigration from South Asia into Central Asia, rather than vice versa. The findings of the present study likewise do not support the Out of India Model, for if this model were true, then there still ought to be affinities between Late Bronze Age Central Asians (now the recipient population) and any of the South Asian

samples, especially those of the Indus Valley and northern Pakistan (the alleged source populations).

Consequently, our findings not only counter the claim of relatively recent gene flow into South Asia from Central Asia, but also suggest the genetic origin of northern Pakistani ethnic groups is likely of a polyphyletic nature. That is, the differentiation seen among members of contemporary ethnic groups of northern Pakistan are likely due to accumulation of mutations in populations separated by geographic, social or temporal isolation. The results also revealed that, when considered on a global scale, members of these ethnic groups are closer in their genetic background to the Europeans than to the East Asians. The lack of affinities with East Asians is intriguing, given recent genetic studies which indicate some affinities between Hazaras (or Chengazis) of Hunza with East Asians, and which some authorities ascribe to gene flow from members of Genghis Khan's army in the 13th century of the Common Era. Indeed, a recent examination of tooth size allocation throughout the permanent dentition among Chengazis from Sakrdu, Gilgit-Baltistan (Camp, 2013) confirmed the lack of affinities to other ethnic groups of northern Pakistan. As such, our results are in agreement with those of Camp and others, which indicate that if such gene flow did occur, it was confined to these Hazara and did not spread to other ethnic groups of northern Pakistan. Thus, it appears that the presence of such hard geographic barriers as the Gobi Desert and lofty Karakoram and Himalayan Mountains between Pakistan and China served as an obstacle to any substantial or long-term gene flow across the regions.

Mitochondrial DNA analysis of people of Northern Pakistan concluded the variable genetic landscape previously reported for this area. Our study for collecting genetic affinities of different ethnic groups of the Districts Abbottabad and Mansehra was the first of its nature in Pakistan. It provided a baseline for future investigation and a handy model for replication with respect to other tribes of the region or elaborating dental and molecular anthropology of the people from different regions of the world. We recommend that other parameter of paternal and maternal DNA should also be employed for bringing more clarity with reference to phylogenetics of the people of the area. Furthermore the subcastes among the major nationalities of the region like Gujar, Kohistani and Patans needs elaboration to provide a clear picture of the caste genetics.

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APPENDIX I

ETHNOGENETIC PROJECT

DEPARTMENT OF GENEITCS
GARDEN CAMPUS HAZARA UNIVERSITY, MANSEHRA
Tel: 0997-414131 Fax: 0997-530046 URL:http//www.hu.edu.pk

NOTE: This performa is the property of the **ETHNO GENETIC PROJECT (HEC)** Department of Genetics Hazara University Mansehra Pakistan. This information will be used for research purpose only and will be kept secret.

Ref. #: _____

Lab code: _____

Date: _____

DETAILS OF PARTICIPANT

Name: _____Father's Name: _____

Age: _____ Sex: _____Native Language:_____

Ethnic Group: _____ Caste _____

Collection point:_____

Home Address: _____

_____

Biological Samples Collected

☐ Dental impressions ☐ Saliva

Consent of Participant

- The researchers have informed me about the purpose of sample collection and their use in human genetics research. I have provided samples voluntarily to improve science in Pakistan.

Signature of Participant: _____

APPENDIX II

THE ASU DENTAL ANTHROPOLOGY SYSTEM

The Arizona State University Dental Anthropology System is used for the analysis of a set of traits that allows us to measure the presence/absence dichotomy and obtain replicability of results among the observers. The ASUDAS uses standard recording forms and 3D reference plaques (Figure 30). The reference plaques enhance the observational precision by giving the researcher a reform for comparison. Although it will probably never be possible to obtain 100% accuracy, gathering 'good' dental morphological data for this research were obtained by scoring the non-metric traits in good lighting with the use of 3D reference plaques. These plaques are a very important part of the methodology. It enables the researcher to get familiar with the different nonmetric traits and reliably compare them for assessing different grades of presence. Furthermore, caution and repeat observations serve to minimize intra observer error. The protocol allows the scientist to analyze dental phenotypes visually assessed non metric dental traits. The goal is thereby to make systematic observations that can be replicated with accuracy. The observations should yield the same results when analyzed by the same researcher on different occasions. They should also coincide closely with the results of other workers examining the same set for the same variables.

Fig.30. Replica of the 3D reference cast for the scoring of shoveling of the UI1

Data should be obtained with a minimum of intraobserver and interobserver error. The study of Nichol and Turner (1986) concluded that the greatest difficulty in making consistent scoring judgments occur at the 'trait threshold', the presence-absence breakpoint. Further, mistakes are often made when defining different grades of expression called 'threshold expressions'. The scoring procedures in the ASU system are focused mainly on the morphological features of the crowns and roots, having special features in function of the type of the root. These simplified sets of morphological features of the crown and roots are obtained from the qualitative scoring proposed by Turner *et al.,* (1991).

APPENDEX III

STOCK REAGENTS

Phenol:Chloroform Mixture (1:1)

For each sample 200uL of phenol and 200uL of chloroform were used.

Lysis Buffer

500mM Tris-base

250 mM EDTA

5% SDS

Proteinase K 75ug/mL of lysis solution

B-mercaptoethanol (14.4M), 1uL/mL of lysis solution

50X TAE buffer

M Tris-HCl pH8

0.5 M EDTA

Make up to 1 L with dH2O and autoclave

Bromophenol blue dye

50 ml dH2O

50 g sucrose

1.86 g EDTA

0.1 g bromophenol blue

Dissolve

Adjust volume to 100 ml with dH2O, stir overnight

pH to 8.0

Filter through Whatmann filter paper

Store at room temperature

10 mg/ml Ethidium bromide (EtBr)

Add 1 g of ethidium bromide to

100 ml of ddH2O

Stir for several hours until completely dissolved

Store wrapped in aluminum foil at 4˚C

1kb size standard

285 µl 1kb ladder (cat# DM001)

143 µl Ficoll dye

2 400 µl 1 X TE

ANNEXURE I

Haplotypes and their respective variants

Sample	Haplotype	SNP Position						
Ab-1	R7	16319A	16362C					
Ab-10	U7a4	16126C	16309G	16318T				
Ab-11	M4	16145A	16223T	16261T	16311C			
Ab-12	H5	16304C						
Ab-14	R7	16319A	16362C					
Ab-15	H3x	16368C						
Ab-16	H2a2a1c	16051G						
Ab-17	D4m2	16042A	16223T	16362C				
Ab-18	M4	16145A	16223T	16261T	16311C			
Ab-19	T2b2	16126C	16294T	16296T				
Ab-2	M4	16145A	16223T	16261T	16311C			
Ab-20	D6	16189C	16223T	16311C	16362C			
Ab-21	H2a3	16274A						
Ab-23	M3	16126C	16223T					
Ab-26	H2a2b1a1	16235G	16291T	16293G	16400T			
Ab-3	T2b2	16126C	16294T	16296T				
Ab-34	M3c2	16126C	16154C	16223T				
Ab-39	F1b1a	16129A	16189C	16232A	16249C	16304C	16311C	16344T
Ab-40	H11a2a	16092C	16140C	16293G	16311C			
Ab-41	R0a1b	16093C	16126C	16189C	16362C			
Ab-42	M4	16145A	16223T	16261T	16311C			
Ab-46	N	16223T						
Ab-5	T2b2	16126C	16294T	16296T				
Ab-6	N	16223T						
Ab-7	H	16129A						
Ab-8	L3'4	16223T	16311C					
Ab-9	H6	16362C	16482G					
Aw-1	H24	16293G						
Aw-10	H6b	16300G	16362C	16482G				
Aw-11	T2b2b	16126C	16294T					
Aw-13	M3	16126C	16223T					
Aw-15	H3a	16239G						
Aw-2	HV2	16217C						
Aw-20	H2a2a1c	16051G						
Aw-21	M3	16126C	16223T					
Aw-23	R6a	16129A	16266T	16274A	16362C			
Aw-24	P6	16311C	16362C					

Aw-25	N	16223T						
Aw-27	M3	16126C	16223T					
Aw-28	T2b2b	16126C	16294T					
Aw-29	L3'4	16223T	16311C					
Aw-3	W6	16192T	16223T	16292T	16325C			
Aw-4	T1	16126C	16163G	16189C	16294T			
Aw-5	H2	16311C						
Aw-6	H	16129A						
Aw-7	M3	16126C	16223T					
Aw-8	H3b1	16129A	16256T					
Aw-9	M3	16126C	16223T					
AwM-1	H6b	16300G	16362C	16482G				
AwM-10	V16	16298C	16301T					
AwM-11	J1c2b	16069T	16093Y	16126C				
AwM-12	N9a4b	16092C	16145A	16172C	16223T	16245T	16257A	16261T
AwM-13	C4a2'3'4	16223T	16298C	16327T	16357C			
AwM-14	U7	16309G	16318T					
AwM-15	U7	16309G	16318T					
AwM-16	H1bt	16527T						
AwM-18	U7	16309G	16318T					
AwM-19	M33a2	16169T	16172C	16223T				
AwM-2	N	16223T						
AwM-20	M33a2	16169T	16172C	16223T				
AwM-21	R5a2	16266T	16304C	16356C	16524G			
AwM-22	U7	16309G	16318T					
AwM-23	T1	16126C	16163G	16189C	16294T			
AwM-24	P6	16311C	16362C					
AwM-25	N	16223T						
AwM-3	U4a1	16134T	16356C					
AwM-4	R5a2	16266T	16304C	16356C	16524G			
AwM-5	X2c	16189C	16223T	16255A	16278T			
AwM-6	H6	16362C	16482G					
AwM-8	N	16223T						
AwM-9	N9a4b	16092C	16145A	16172C	16223T	16245T	16257A	16261T
Guj-1	H1ar	16468C						
Guj-11	S1	16075C	16223T					
Guj-12	H2a2b	16235G	16291T					
Guj-13	H2a2a1c	16051G						
Guj-14	H	16129A						
Guj-15	X	16189C	16223T	16278T				
Guj-16	H2a2b	16235G	16291T					

Guj-17	H2a1	16354T						
Guj-19	H2a2a1c	16051G						
Guj-20	H2a1	16354T						
Guj-21	H2a2a1c	16051G						
Guj-22	H2a2a1c	16051G						
Guj-23	H5	16304C						
Guj-24	J1b5b	16069T	16126C	16145A	16222T	16261T	16290T	
Guj-25	H13b	16362C						
Guj-26	H2a2a1c	16051G						
Guj-27	H2	16311C						
Guj-28	W6	16192T	16223T	16292T	16325C			
Guj-29	H5a1g1	16172C	16304C	16311C				
Guj-3	H2a2a1c	16051G						
Guj-32	U7	16309G	16318T					
Guj-34	H5	16304C						
Guj-36	M4	16145A	16223T	16261T	16311C			
Guj-38	H5	16304C						
Guj-39	N	16223T						
Guj-4	H2a2a1c	16051G						
Guj-40	H6a1a2a	16362C						
Guj-41	R5a2	16266T	16304C	16356C	16524G			
Guj-42	T2b	16126C	16294T	16296T	16304C			
Guj-43	N	16223T						
Guj-44	R0a	16126C	16362C					
Guj-45	W6	16192T	16223T	16292T	16325C			
Guj-46	N	16223T						
Guj-47	H13b	16362C						
Guj-48	M3c2	16126C	16154C	16223T				
Guj-49	G1b	16129A	16223T					
Guj-5	H13b	16362C						
Guj-6	H2a2a1c	16051G						
Guj-9	M5a2a2	16129A	16144A	16223T				
Jad-1	D4b1b1a	16223T	16287T	16319A	16362C	16399G		
Jad-11	H1c3b	16189C	16362C					
Jad-13	D	16189C	16223T	16362C				
Jad-14	H2a2b	16235G	16291T					
Jad-15	I1	16129A	16223T	16311C	16391A			
Jad-16	H2a2a1c	16051G						
Jad-17	H6a1a2a	16362C						
Jad-18	H1ab1	16189C	16234T					
Jad-2	R5a	16266T	16304C	16524G				

Jad-20	I1	16129A	16223T	16311C	16391A			
Jad-21	H1	16239T						
Jad-22	G1b	16129A	16223T					
Jad-23	M5a2a1a	16129A	16223T	16265C				
Jad-25	H1ar	16468C						
Jad-3	H5	16304C	16311C					
Jad-8	H1b1	16189C	16356C	16362C				
kar-1	H2	16311C						
kar-10	X2e2b	16189C	16223T	16265G	16278T			
kar-11	N1a3	16201T	16223T	16265G				
kar-12	S1	16075C	16223T					
kar-13	M33c	16111T	16223T	16362C				
kar-14	E2	16051G	16223T	16362C	16390A			
kar-15	H	16129A						
kar-16	H1	16239T						
kar-17	H2a2a1c	16051G						
kar-18	N1a3	16201T	16223T	16265G				
kar-19	A2	16223T	16290T	16319A	16362C			
kar-2	G1b	16129A	16223T					
kar-20	P6	16311C	16362C					
kar-21	M33c	16111T	16223T	16362C				
kar-22	F1c1a	16111T	16129A	16304C				
kar-23	M33c	16111T	16223T	16362C				
kar-24	M4	16145A	16223T	16261T	16311C			
kar-3	L3a	16223T	16316G					
kar-4	H1	16239T						
kar-5	H1	16239T						
kar-6	N	16223T						
kar-7	X	16189C	16223T	16278T				
kar-8	N1a3	16201T	16223T	16265G				
kar-9	N	16223T						
syd-10	M5b2	16048A	16129A	16223T				
syd-11	H5e	16294T	16304C					
syd-12	H3x	16368C						
syd-13	H2	16311C						
syd-15	U7	16309G	16318T					
syd-16	H6a1a2a	16362C						
syd-17	H2	16311C						
syd-18	M5a2a1a	16129A	16223T	16265C				
syd-2	H1ar	16468C						
syd-24	H13b	16362C						

syd-25	J1b1a2	16069T	16126C	16145A	16187T	16222T	16261T	
syd-26	D6	16189C	16223T	16311C	16362C			
syd-27	H13b	16362C						
syd-28	D4e5b	16223T	16274A	16291T	16362C			
syd-3	M30c1	16166-	16223T					
syd-31	H3p	16222T						
syd-32	H2	16311C						
syd-33	H17a1	16129A	16291T					
syd-34	N11a1	16189C	16223T	16355T				
syd-35	HV2	16217C						
syd-36	U7a4	16126C	16309G	16318T				
syd-37	H6a1a2a	16362C						
syd-38	H1	16239T						
syd-39	H6a1a2a	16362C						
syd-4	M3	16126C	16223T					
syd-40	HV2	16217C						
syd-43	M4	16145A	16223T	16261T	16311C			
syd-44	H13b	16362C						
syd-5	M3	16126C	16223T					
syd-6	H6a1a2a	16362C						
syd-7	L3'4	16223T	16311C					
syd-8	M30c1	16166-	16223T					
syd-9	U7	16309G	16318T					
Tan-10	M73'79	16223T	16278T					
Tan-11	J1b	16069T	16126C	16145A	16222T	16261T		
Tan-12	M49	16223T	16234T					
Tan-13	M49	16223T	16234T					
Tan-14	M73'79	16223T	16278T					
Tan-15	X	16189C	16223T	16278T				
Tan-16	T2b2b	16126C	16294T					
Tan-17	H1ar	16468C						
Tan-18	H2a2b	16235G	16291T					
Tan-19	M4	16145A	16223T	16261T	16311C			
Tan-2	H	16129A						
Tan-20	N	16223T						
Tan-21	H	16129A						
Tan-22	L3'4	16223T	16311C					
Tan-23	H2a2a1c	16051G						
Tan-25	E2	16051G	16223T	16362C	16390A			
Tan-28	M3c2	16126C	16154C	16223T				
Tan-29	H13b	16362C						

Tan-3	H8	16288C	16362C					
Tan-30	L4	16223T	16311C	16362C				
Tan-31	H1bt	16527T						
Tan-32	M18	16223T	16318T					
Tan-34	U7	16309G	16318T					
Tan-36	M30c1	16166-	16223T					
Tan-37	N	16223T						
Tan-38	H2	16311C						
Tan-39	U7	16309G	16318T					
Tan-4	H5	16304C	16311C					
Tan-40	M5a2a1a	16129A	16223T	16265C				
Tan-41	X	16189C	16223T	16278T				
Tan-42	L3d3a	16124C	16189C	16223T	16278T	16304C	16311C	
Tan-43	S1	16075C	16223T					
Tan-44	N	16223T						
Tan-45	H1aj1	16192T						
Tan-46	H6	16362C	16482G					
Tan-47	HV2	16217C						
Tan-49	H2a2a1d	16172C						
Tan-5	H	16129A						
Tan-6	HV2	16217C						
Tan-7	R5a2	16266T	16304C	16356C	16524G			

ANNEXURE II

Haplotypes and their respective variants obtained through mtDNA HVSII analysis

Sample ID	Haplotype	SNP Position
J22	A4	73G 152C 200G 235G 263G
A1	M34	73G 263G 489C 569T
A10	HV2	73G 152C 195C 263G
A11	H32	73G 152C 263G
A12	HV2	73G 152C 195C 263G
A13	M3c	73G 152C 263G 482C 489C
A14	H2a2a2	152C 263G
A15	X2d1	73G 195C 204C 207A 263G
A17	H32	73G 152C 263G
A18	H6	239C 263G
A2	T2d1b	73G 152C 194T 200G 263G
A20	M30a	73G 195A 263G 489C 513A
A21	H6	239C 263G
A22	U4a2	73G 195C 263G 310C 499A
A23	M3	73G 263G 482C 489C
A24	HV2	73G 152C 195C 263G
A25	HV2	73G 152C 195C 263G
A26	M33a2a	73G 150T 263G 462T 489C
A27	HV2	73G 152C 195C 263G
A28	M44	73G 146C 263G 489C
A29	M30b	73G 152C 195A 263G 489C
A3	U2b2	73G 146C 152C 234G 263G
A30	H2a2a2	152C 263G
A31	H32	73G 152C 263G
A32	R11	73G 185A 189G 263G
A34	H32	73G 152C 263G
A35	W4	73G 143A 189G 194T 195C 196C 204C 207A 263G
A36	H6	239C 263G
A37	M30b	73G 152C 195A 263G 489C
A38	M30b	73G 152C 195A 263G 489C
A39	H32	73G 152C 263G
A4	R11	73G 185A 189G 263G
A40	R2	73G 146C 152C 263G
A41	H2a2a2	152C 263G
A42	J1c16	73G 152C 185A 228A 263G 295T 462T 489C

A44	H5a3	263G 456T 513A
A45	H32	73G 152C 263G
A46	R30b1	73G 152C 263G 299- 373G 480C
A47	H32	73G 152C 263G
A48	H	146C 195C 263G
A5	M3c	73G 152C 263G 482C 489C
A50	M35	73G 199C 263G 489C
A6	M3	73G 263G 482C 489C
A7	M	73G 263G 489C
A8	X2d	73G 195C 263G
A9	M65	73G 263G 489C 511T
AA1	M3c	73G 152C 263G 482C 489C
AA10	H32	73G 152C 263G
AA11	W	73G 189G 194T 195C 204C 207A 263G
AA12	M30	73G 195A 263G 489C
AA13	M30	73G 195A 263G 489C
AA14	H2a2a	263G
AA15	J1b1b	73G 263G 271T 295T 462T 489C
AA16	M30b	73G 152C 195A 263G 489C
AA17	M30b	73G 152C 195A 263G 489C
AA18	M3c	73G 152C 263G 482C 489C
AA19	U2b2	73G 146C 152C 234G 263G
AA2	R24	73G 146C 263G
AA20	U2b2	73G 146C 152C 234G 263G
AA21	H5a3	263G 456T 513A
AA22	U1a1	73G 263G 285T 385G
AA23	N1a1'2	73G 199C 204C 263G
AA25	M34	73G 263G 489C 569T
AA26	M30	73G 195A 263G 489C
AA27	M13'46'61	73G 152C 263G 489C
AA28	H32	73G 152C 263G
AA29	M30	73G 195A 263G 489C
AA3	M65	73G 263G 489C 511T
AA30	X2d	73G 195C 263G
AA31	H32	73G 152C 263G
AA32	X2d	73G 195C 263G
AA33	M34	73G 263G 489C 569T
AA34	H32	73G 152C 263G
AA35	H2a2a2	152C 263G

AA36	W	73G 189G 194T 195C 204C 207A 263G
AA37	H32	73G 152C 263G
AA38	X2d	73G 195C 263G
AA39	M13'46'61	73G 152C 263G 489C
AA4	X2d	73G 195C 263G
AA40	M34	73G 263G 489C 569T
AA41	R6a1	73G 195C 228A 263G
AA42	R6a1	73G 195C 228A 263G
AA43	M30	73G 195A 263G 489C
AA44	U2b2	73G 146C 152C 234G 263G
AA45	M34	73G 263G 489C 569T
AA46	X2d	73G 195C 263G
AA47	H2a2a2	152C 263G
AA48	H1an2	151T 152C 263G
AA49	W	73G 189G 194T 195C 204C 207A 263G
AA5	X2d	73G 195C 263G
AA50	M30a	73G 195A 263G 489C 513A
AA6	M30	73G 195A 263G 489C
AA7	U2e1	73G 152C 217C 263G 340T 508G
AA8	H32	73G 152C 263G
AA9	M13'46'61	73G 152C 263G 489C
G1	M37	73G 151T 152C 263G 489C
G10	W	73G 189G 194T 195C 204C 207A 263G
G11	R30b	73G 152C 263G 373G
G12	M71a1	73G 150T 263G 489C
G13	M65	73G 263G 489C 511T
G14	M39b1	66T 73G 153G 263G 463T 485C 489C
G15	M39b1	66T 73G 153G 263G 463T 485C 489C
G16	M65	73G 263G 489C 511T
G17	H2a2a2	152C 263G
G18	X1'3	73G 146C 153G 263G
G19	M39b1	66T 73G 153G 263G 463T 485C 489C
G2	M35	73G 199C 263G 489C
G20	J1d	73G 152C 263G 295T 462T 489C
G21	R30b	73G 152C 263G 373G
G22	M	73G 263G 489C
G23	U2e1	73G 152C 217C 263G 340T 508G
G27	M39b1	66T 73G 153G 263G 463T 485
G28	M30	73G 195A 263G 489C

G29	H3	73G 263G
G3	F1d	73G 146C 249- 263G
G30	H32	73G 152C 263G
G31	R30b	73G 152C 263G 373G
G32	M37	73G 151T 152C 263G 489C
G33	H2a2a	263G
G34	U4a3	73G 195C 247A 263G 499A
G35	M30b	73G 152C 195A 263G 489C
G37	H32	73G 152C 263G
G38	M30	73G 195A 263G 489C
G39	H32	73G 152C 263G
G4	H3	73G 263G
G40	U4a1	73G 152C 195C 263G 499A
G41	H32	73G 152C 263G
G42	M30b	73G 152C 195A 263G 489C
G43	U2e1'2'3	73G 152C 217C 263G 508G
G44	K1e	73G 152C 263G 524.1A 524.2C
G45	M14	73G 234G 263G 489C
G46	M	73G 263G 489C
G47	X2d1	73G 195C 204C 207A 263G
G48	N1a1'2	73G 199C 204C 263G
G49	U2e1	73G 152C 217C 263G 340T 508G
G5	M3	73G 263G 482C 489C
G50	M3	73G 263G 482C 489C
G6	M39b1	66T 73G 153G 263G 463T 485C 489C
G7	U2e1'2'3	73G 152C 217C 263G 508G
G8	H32	73G 152C 263G
G9	H32	73G 152C 263G
J1	I1	73G 199C 204C 250C 263G 455.1T 573.1C
J10	M30	73G 195A 263G 489C
J11	H15	55C 57C 263G
J12	X2d	73G 195C 263G
J13	H32	73G 152C 263G
J15	H2a2a	263G
J16	X2d	73G 195C 263G
J17	J	73G 263G 295T 489C
J18	M34	73G 263G 489C 569T
J19	H2a2a	263G
J2	R5a1	73G 93G 200G 263G

J20	M34	73G 263G 489C 569T
J21	R30b1	73G 152C 263G 299- 373G 480C
J23	U2e1	73G 152C 217C 263G 340T 508G
J24	H1an2	151T 152C 263G
J25	M30	73G 195A 263G 489C
J26	H32	73G 152C 263G
J27	M65	73G 263G 489C 511T
J28	M3c	73G 152C 263G 482C 489C
J29	U2e1	73G 152C 217C 263G 340T 508G
J3	I1	73G 199C 204C 250C 263G 455.1T 573.1C
J30	H32	73G 152C 263G
J31	W	73G 189G 194T 195C 204C 207A 263G
J32	M3	73G 263G 482C 489C
J33	M30	73G 195A 263G 489C
J34	H3	73G 263G
J35	H32	73G 152C 263G
J36	X2d	73G 195C 263G
J37	M3	73G 263G 482C 489C
J38	H3	73G 263G
J39	H32	73G 152C 263G
J4	U2e1	73G 152C 217C 263G 340T 508G
J40	H3	73G 263G
J41	Z3a	73G 152C 207A 249- 263G 489C
J42	M65	73G 263G 489C 511T
J43	M34	73G 263G 489C 569T
J44	N2	73G 189G 263G
J45	HV0	72C 195C 263G
J46	H32	73G 152C 263G
J47	U4'9	73G 195C 263G 499A
J48	X2d	73G 195C 263G
J49	M34	73G 263G 489C 569T
J5	K1e	73G 152C 263G 524.1A 524.2C
J8	I1	73G 199C 204C 250C 263G 455.1T 573.1C
J9	H15a1b	55C 57C 146C 263G
K1	H2a2a2	152C 263G
K10	K2a8	73G 146C 152C 207A 263G
K11	K2a8	73G 146C 152C 207A 263G
K12	M6	73G 263G 461T 489C
K13	M70	73G 236C 263G 489C

K14	M34	73G 263G 489C 569T
K15	U2b2	73G 146C 152C 234G 263G
K16	U2b2	73G 146C 152C 234G 263G
K17	U2b2	73G 146C 152C 234G 263G
K18	N1a3	73G 189G 195C 204C 207A 210G 263G
K19	A4	73G 152C 200G 235G 263G
K2	N2	73G 189G 263G
K20	N1a3	73G 189G 195C 204C 207A 210G 263G
K22	M30b	73G 152C 195A 263G 489C
K23	M	73G 263G 489C
K24	W	73G 189G 194T 195C 204C 207A 263G
K26	M13'46'61	73G 152C 263G 489C
K28	U3	73G 150T 263G
K3	M44	73G 146C 263G 489C
K30	HV2a	73G 152C 195C 246C 263G 573.1C
K31	M34	73G 263G 489C 569T
K32	A4	73G 152C 200G 235G 263G
K33	R6a1	73G 195C 228A 263G
K34	H32	73G 152C 263G
K35	H32	73G 152C 263G
K36	M34	73G 263G 489C 569T
K4	U2b2	73G 146C 152C 234G 263G
K5	H32	73G 152C 263G
K6	X2d1	73G 195C 204C 207A 263G
K7	M30b	73G 152C 195A 263G 489C
K8	N1a3	73G 189G 195C 204C 207A 210G 263G
K9	X2d1	73G 195C 204C 207A 263G
S1	M2a1a	73G 195C 204C 263G 447G 489C
S10	R24	73G 146C 263G
S11	H32	73G 152C 263G
S12	M3	73G 263G 482C 489C
S14	H32	73G 152C 263G
S15	M30c	73G 146C 195A 263G 489C
S16	M3	73G 263G 482C 489C
S17	H32	73G 152C 263G
S18	H32	73G 152C 263G
S19	H32	73G 152C 263G
S2	M3	73G 263G 482C 489C
S20	J1c3i	73G 228A 263G 295T 462T 489C

S21	K2a5	73G 146C 152C 263G 324T
S22	M34	73G 263G 489C 569T
S24	H3	73G 263G
S26	H32	73G 152C 263G
S27	M34	73G 263G 489C 569T
S28	W	73G 189G 194T 195C 204C 207A 263G
S29	W	73G 189G 194T 195C 204C 207A 263G
S3	M2a1a	73G 195C 204C 263G 447G 489C
S30	H32	73G 152C 263G
S31	H32	73G 152C 263G
S32	H	146C 195C 263G
S33	H32	73G 152C 263G
S34	H32	73G 152C 263G
S35	H1c	152C 263G 477C
S36	M13'46'61	73G 152C 263G 489C
S37	H32	73G 152C 263G
S38	M3	73G 263G 482C 489C
S39	M3c	73G 152C 263G 482C 489C
S40	H32	73G 152C 263G
S41	H32	73G 152C 263G
S42	H3	73G 263G
S43	H2a2a2	152C 263G
S44	H32	73G 152C 263G
S47	H2a2a	263G
S6	M3	73G 263G 482C 489C
S7	H57	64T 263G
T1	H32	73G 152C 263G
T10	M3c	73G 152C 263G 482C 489C
T11	M70	73G 236C 263G 489C
T12	H32	73G 152C 263G
T13	HV0f	195C 263G
T14	W3b	73G 189G 194T 195C 199C 204C 207A 263G
T15	H32	73G 152C 263G
T16	W4	73G 143A 189G 194T 195C 196C 204C 207A 263G
T17	R30b1	73G 152C 263G 299- 373G 480C
T18	M18a	73G 93G 194T 246C 263G 489C
T19	M30c	73G 146C 195A 263G 489C
T2	R0a4	58C 64T 150T 263G
T20	M30a	73G 195A 263G 489C 513A

T23	H32	73G 152C 263G
T24	H32	73G 152C 263G
T25	M30c	73G 146C 195A 263G 489C
T26	K1e	73G 152C 263G 524.1A 524.2C
T27	HV2	73G 152C 195C 263G
T3	J2a2	73G 150T 195C 263G 295T 489C
T30	H2a2a	263G
T31	R2	73G 146C 152C 263G
T32	H32	73G 152C 263G
T33	H2a2a	263G
T34	H2a2a2	152C 263G
T35	H2a2a	263G
T36	H2a2a	263G
T38	H3	73G 263G
T39	W3b	73G 189G 194T 195C 199C 204C 207A 263G
T4	M13'46'61	73G 152C 263G 489C
T40	N1a	73G 204C 263G
T42	H1av1	150T 263G
T43	M3	73G 263G 482C 489C
T46	M13'46'61	73G 152C 263G 489C
T47	M3	73G 263G 482C 489C
T49	R30b	73G 152C 263G 373G
T5	H32	73G 152C 263G
T50	H32	73G 152C 263G
T51	J1c3i	73G 228A 263G 295T 462T 489C
T6	H32	73G 152C 263G
T7	H3	73G 263G
T8	H32	73G 152C 263G
T9	M30b	73G 152C 195A 263G 489C