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Telomere: A Forensic Approach For Age Estimation

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ABSTRACT

In forensics, it is very important to know the identity and age of an individual. It is known fact that without proper evidence nobody is found guilty in the court of law. Evidences are collected at the crime scene and most of the time crime scene is reconstructed to give idea how the crime has occurred and establish the relation with the crime and the suspect. In order to establish the involvement of suspect, the biological sample like blood, blood stain, semen, seminal stain, saliva, hair, tissue etc. which were found at the crime scene, help to establish the individual delinquent. Thus, these biological samples are very useful tool for identification of an individual as well as can be used to find the age of an individual. Age estimation is one of the parameter for identification in both living and dead human beings, using molecular biology techniques. Age can be estimated by the biological samples to find the blueprint of criminal in the absence of reference sample for identification. In present time, age estimation is done by radiography and anthropometry which require bone, skull and teeth. However, age can be estimated by molecular techniques i.e. the southern blot method or RT-PCR method. But of these, RT-PCR method is simple, fast, reliable and has high output of samples quantity in comparison to the southern blot method. DNA can be amplified even with the degraded sample with the help of RT-PCR technique.

Keywords: Telomere, Age estimation, RT-PCR, Southern blot

INTRODUCTION

In forensic science, investigation of crime scene plays a pivotal role in questioning how the crime has taken place, the motive, type of weapon used, who was involved, time of occurrence of crime

and many other relevant things. Moreover, according to Locard's exchange principle there are some minute samples exchanged by suspect or victim which many a times leads to give the exclusive clues about the crime. There are some biological samples which have no morphological value even though are important for identification of an individual present at crime scene. In such situations, the best method to identify an individual is by DNA profiling from the biological samples collected at the scene of crime

The biological samples help to identify the person if there is the data bank[8] of all the people living in a country or by comparing the DNA profile of those biological samples collected at the crime scene with known reference samples. But when there is no such type of reference sample or data bank (as in India), it is difficult to identify an individual. The best option left there is to subject the suspect to the latest technique i.e. DNA analysis[9]. The most important issue raised in many crime cases is the age of an individual whose particulars are not known. In general, the age estimation is one of the parameter for identification in both living and dead human beings, using molecular biology technique. Therefore, it is of prime importance to find out the age of an individual so that the crime is correlated with the suspects involved in the said crime. In present time, estimation of the human age for forensic purpose depends on the radiography and anthropometry[17]. For this bone, skull and teeth samples or dental records[21,27] are required for the estimation of human age, but most of the time other biological samples like blood, blood stain, semen, semen stain, saliva, hair, tissue etc are found on the crime scene. In this situation, the conventional methods cannot be used for the accurate age estimation of an individual but with the use of molecular techniques[1], the estimation of age of an individual is possible. Thus the age of individual can be estimated using molecular based technique[3]-using Mitochondrial DNA deletion, Telomere shortening, Aspartic acid racemisation and advanced glycation end product.

Therefore, at molecular level, telomere plays an important role for the estimation of human age especially for forensic purpose from the above mentioned biological samples. Age can be estimated by measuring relative telomere length of the individual. It is very fast and reliable method and achieves both targets i.e. age and identification of the person with same sample or DNA extracted from the biological sample found on the crime scene.

TELOMERE

Telomere in humans is a region of repetitive nucleotide sequence composed of tandem repeats TTAGGG which are found at each end of chromosome i.e. long arm and short arm, as it prevent the chromosome pair to fuse with the neighbouring chromosome. At the time of replication, the length of chromosome reduces as some telomere fragment cannot be copied due to certain enzymes that help in duplication of DNA so it cannot duplicate at the end of chromosome on lagging strand[7]. In every replication, telomere length gets reduced up to 50–100 base pairs and finally reaches to critical stage, where it gets cellular senescence. Genomic stability is maintained by telomeres as it protects the DNA from damage at the ends of chromosome[14,19].

TELOMERE LENGTH AND AGE

Telomere length is inversely proportional to the age of individual i.e. as telomere length decreases the age increases. So telomere length would be used as the parameter for age estimation for forensic purpose.

Various studies have been carried on telomere length measurement by using real time PCR method and southern blot method in different countries i.e. Japan, china (Tibetan), Sweden, Australia, Germany, Netherland, Thai, Taiwan, and Egypt. In these studies, different biological samples i.e. peripheral blood, blood stain, cadaver blood, tooth pulp, buccal cell etc have been used followed by DNA extraction from these sample with organic method or automatic extraction method, after that subjected to RT-PCR method or southern blot method to find the relative telomere length and establish the correlation between telomere length and the age of individual[7]. It is also found from these studies that telomere length varies in male and female individual.

TELOMERE LENGTH MEASUREMENT

The length of telomere cannot be measured directly as there is no reliable method to measure it. Following techniques are generally used to measure telomere length:

- Terminal Restriction Fragment (TRF) method by southern blot.
- Histochemical method (Q-FISH).
- Real-Time PCR method.
- Flow-FISH method

TELOMERE LENGTH MEASUREMENT BY SOUTHERN BLOT

Telomere was used as a parameter for age estimation by Tsuji[2] *et al.* on Japanese population using southern blot method, where first hybridization was done by using radioactive 32P-(TTAGGG) n oligo nucleotide probe and then undergo digestion with Hinf / Rsa I and then genomic DNA fragments were embedded on a nylon membrane and exposed to auto radiographic film.

They found that the length of telomere decreases with the age. They observed in their study which was conducted on 60 healthy individual aged between 0-85 years in Japan that average terminal restriction fragment length clearly shows a tendency with aging “r” = -0.8320 ($P < 0.01$),with regression analysis R^2=0.6922 between TL and age but standard error for age prediction was ± 7.037 quoted.

Further, they also demonstrated that mean Telomere length of dried blood stains stored at room temperature for 5 months was 500 bp which was shorter than that of freshly drawn blood samples from the same individuals. These results may be due to the use of absolute quantitation of Terminal Restricted Fragment length. This study shows that the length of telomere decreases with age within the same individual when stored the same sample for five months.

The same result was observed by Takasaki[29] *et al.* in their study which was based on 100 individual (16-70 years) using molar-tooth pulp in Japan. The average terminal restriction fragment length of dental pulp clearly shortens with aging “r” = -0.749 ($P < 0.01$), with regression analysis R^2=0.562 between TL and age but there was ± 7.52 standard error for age prediction.

Further, Fu Ren[5] *et al.* also demonstrated the same result using southern blot method based on 105 healthy individual (0-81 years) in China (Tibetans). The mean Terminal Restriction Fragment length was inversely correlated with age “r” = -0.913 ($P < 0.01$), and sex of the individual must be considered when age was estimated by telomere method with regression analysis R^2=0.815 between TL and age but standard error for age prediction was found ± 9.832.

The study carried on 100 Thai cadaveric subjects by Srettabunjong[28] *et al.* concluded in their study using southern blotting method that mean Terminal Restriction Fragment length was inversely correlated with age "r" = -0.625 (P < 0.001), with regression analysis R^2=0.391 between TL and age but standard error for age prediction was ±9.604. Kimara[15] *et al.* also found the same results that telomere length decrease with age. These results were same as it was found by Guan[11] *et al.* who confirmed that the length of telomeres decreased with age, they also found that mean TRF length is shortened by 77bp/year in overall study.

TELOMERE LENGTH MEASUREMENT BY RT-PCR

The length of telomere was estimated by RT PCR which was started by R.M.Cawthon[22,23] in 2002. He used RT PCR and southern blot method simultaneously on 95 Utah individuals (5-94 years), and found that this method was simple, fast, and reliable and quantative to get high output of samples as compared to the southern blot method and also there is a chance that DNA can be amplified even with the degraded samples[1,24]. In this method, telomere length can be determined by the Telomere-to-Single Copy Gene (T/S) ratio, which is demonstrated to be proportional to the average telomere length in a cell. R.M. Cawthon[22,23] found the same results as it was in southern blot method that telomere length decreases with increase in age.

He also measured the telomere length by monochrome multiplex PCR method in 2008 on same samples that are well preserved. It was observed that with increase in age, telomere length decreased and this multiplex PCR method was more accurate than singleplex PCR method that was adopted earlier.

Alhusseini[18] *et al.* observed in their study that age estimation by relative telomere length by RT-PCR method is a useful method and relative Telomere length shortens with age with correlation coefficient "r" = -0.903 ($p<0.001$) and regression analysis R^2=0.815 between relative TL and age but standard error for age prediction was ±10.14 which is considerably high so it would be used as a complementary method for age estimation. He conducted his study on 80 individuals (1-79 years) in Egypt.

The same result was observed by Hewakapuge[26] *et al.* which was based on telomere length measured in buccal cells of 167 individuals (1-96 years) using quantative RT- PCR method in Australia. They reported in their studies that telomere length decreased with age and found correlation coefficient r = -0.185 ($p<0.05$) and regression R^2=0.03679; between telomere length and age. He also interpreted the cause of differences in the observed "r" values in different studies which was mainly due to differences in ethnicity, sample size and laboratory conditions. They also concluded that different methods used for measuring telomere length have no remarkable impact on telomere length. Zubakov[4] *et al.* in Netherlands, also observed in their study that age estimation by relative telomere length by RT-PCR method is a useful method in the field of forensic science and relative Telomere length shortens with age with correlation coefficient "r" = -0.987 ($p<0.01$) and regression analysis R^2=0.836 between relative TL and age but standard error for age prediction was ±8.9 years.

The standard error for age prediction was quite high in these studies as some would suggest that it will very useful tool for age estimation in forensic while others suggest that it would not help for age estimation. Alhusseini[18] *et al.* and Zubakov[4] *et al.* suggested that RT PCR method may be used for age prediction especially when there was no morphologic information in biological sample as it would provide a rough idea about age of the individual.

Telomere and Gender

The length of telomere also depends on the gender of the individual. In most of the studies telomere length can differ in male and female populations. Fu Ren[5] *et al.* observed in his study that for age estimation one should consider the gender of the individual and they determined that telomere length in the females was considerably longer than males. The same phenomenon was found by Alhusseini[18] *et al.* in their research that the relative telomere length was found longer in females as compared to males. Nordfjall[13] *et al.* in Sweden found that the loss of telomeres base pair was slow in females (16 bp/year) when it was compared to males (25 bp/year), while Hoffmann[10] *et al.* in Germany cited the reason behind longer telomere length in females which was due to presence of estrogens receptor expression in them and that's why men have shorter telomeres as compared to the females. Effros[20] *et al.* also mentioned the reason of longer female telomere length in their study as they found that telomerase activity was increased remarkably when T-lymphocytes was treated with estrogens and resulting in prevention of telomeres from rapid shortening in females. Kimara[15] *et al.* also found females have longer telomere length than males While Guan[11] *et al.* found female has slow loss of telomere i.e. 54bp/year in comparison to male 103bp/year but it was reverse in females when they were attaining menopause (after 50 years) i.e. 46bp/year to 210bp/year. The rate of loss of telomere was more rapid in females after attaining menopause. On the other hand, Srettabunjong[28] *et al.* found that there was no statistically significant difference in male and female mean TRF length.

Here is a summary of different studies done by various researchers in different country with their result:- y= avg. TRF length: bp, a=mean TRF length, X = relative TL

STUDY	POPULATION	METHOD	CORRELATION COFF.	FORMULA (AGE)	R^2	S. ERROR
Alhusseini[18] *et al.*	Egypt	RTPCR	-0.0903(p<0.001)	age= 66.9 – 28 * X	R^2=0.815	±10.14
Hewakapuge[26] *et al.*	Australia	RTPCR	-0.185(p<0.05)	age=relativeTL–1.5/-0.005;	R^2=0.03679	
Zubakov[4] *et al.*	Netherlands	RTPCR	-0.987 (p<0.01)		R^2 = 0.836	±8.9
Srettabunjong[28] *et al.*	Thailand	Southern blot	-0.625(p<0.05)	age=-0.0012a + 113.538	R^2=0.391	±9.604
Fu Ren[5] *et al.*	Tibet	Southern blot	-0.913(p<0.01)	age=-16.539a + 236.287	R^2=0.815	± 9.832.
Takasaki[29] *et al.*	Japanese	Southern blot	-0.749 (P < 0.01)	age= -0.0119y + 168	R^2=0.562	± 7.52
Tsuji[2] *et al.*	Japanese	Southern blot	-0.8320 (P < 0.01)	age= -0.0095y + 148.9	R^2=0.6922	± 7.037.

Telomere length and endogenous factors

Shortening of telomere length can occur due to various endogenous factors present in and around the individual. It includes genetic, pathological and physiological conditions. Various studies have been carried out in which chronic diseases like coronary heart disease, hypertension, diabetes,

liver disease and oxidative stress can affect telomere length. Karlsson[1] *et al.* stated that various biological factors are affecting the Telomere length which includes various diseases, age, sex, lifestyle of the individual. The telomerase activity and inherited telomere length also affect the telomere length. Also Brouilette[25] *et al.* found in his study that oxidative stress increase the rate of telomere length shortening and various psychological disorders, diabetes, inflammation and vascular diseases were associated with it. Saeed[16] *et al.* found in their study that telomere shortening occurs due to various diseases but the aetiology was not known. Salpea[12] *et al.* found in their study that in type 2 diabetes individual has shorter telomere length. They found the evidences about between oxidative stress and reactive oxygen species produced by mitochondrial as overproduction of reactive oxygen species which causes cell death so telomere length might be useful for biological age and cell oxidative damage.

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