CHAPTER - 5

AVERAGE TELOMERIC LENGTH IN THE PERIPHERAL LYMPHOCYTES IN AGING HUMAN SUBJECTS OF DIFFERENT NUTRITIONAL STATUS AND OF DOWN SYNDROME PATIENTS
AVERAGE TELOMERIC LENGTH [ATL] IN THE PERIPHERAL LYMPHOCYTES IN AGING HUMAN SUBJECTS OF DIFFERENT NUTRITIONAL STATUS AND OF DOWN SYNDROME PATIENTS.

One of the biological phenomena that has captured the serious attention of not only scientists but also of sociologists and Governments during the past 20 years, is Aging. As already outlined in Chapter 1 of this thesis, many theories have been proposed to explain the molecular basis of the aging phenomenon (Kanungo, 1994).

During the past five years a new concept has emerged which adds credibility to the aging theories based on the genetic involvement. This new concept not only explains the transformation of somatic cells to malignant but also explains the onset of replicative senescence. This hypothesis which can be termed as ‘telomeric hypothesis of aging and immortality’ is essentially based on the results suggesting that the non-coding repetitive genomic DNA (telomere) located at the tips of the eukaryotic chromosomes may have a telling role in DNA-replication (cell replication) and therefore in the phenomenon of cancer as well as aging.

Telomeres, consisting of a repetitive DNA of a discrete sequence at the ends of the chromosomes are thought to provide stability to the structure of the chromosome (Blackburn and Szostak, 1984; Day et al, 1993). Telomeres also carry out another function in cells. They ensure that during the replication, the end of the linear DNA is replicated completely without any loss of terminal bases at 5'-end. Such a loss is predicted as a natural consequence of the properties of replicative machinery of conventional semiconservative replication (Olovnikov, 1973). The lost sequences of the telomere at each round of replication are synthesised again by an enzyme telomerase (Harley, 1995). If this enzyme is not present in the replicating cells, as is the case with most somatic cells (Rao,
then each round of replication will result in shortening of the telomeric length. When this length reaches a critical level then the cells stop dividing and senescence sets in. However, tissues and cells that have unlimited potential to replicate, for example, germ cells, cancer cells, immortalised cells etc., are found to have either longer or stabilised telomeric lengths and in such cases the activity of telomerase is always found to be present (Delange et al., 1990).

Indeed, reduction in telomeric length has been correlated with the cellular aging. Cells with longer telomeres are found to be capable of undergoing more divisions than those with shorter telomeres (Harley et al., 1990; Allsopp et al., 1992). Furthermore, telomeres are shorter in older individuals than the younger individuals (Hastie et al., 1990; Lindsey et al., 1991; Allsopp et al., 1992; Vaziri et al., 1993).

From all this it is clear now that in eukaryotes, the telomeric length has an intimate role to play in signalling the cell either to enter senescence period or that of immortality.

In chapter 3 it has been presented that chronic restriction of dietary calorie consumption could sustain better DNA-repair capacity, which might lead to improved longevity. If this were to be the case then it would be interesting to see what would be the connection between improved longevity and the telomeric length in such individuals (persons with low and high Body Mass Index).

Similarly, in chapter 4, the results indicated that DNA-repair capacity is at a low level and deteriorates more rapidly in Down syndrome subjects in comparison with normal subjects. Since Down syndrome is typical example of accelerated aging, it would be of interest to examine the telomeric lengths in these subjects.
Therefore a preliminary study has been undertaken to examine the average telomeric length in the peripheral lymphocytes of normal, low BMI and Down syndrome subjects.

**MATERIAL AND METHODS**

Selection of the human subjects of various categories including Down syndrome patients has already been described in chapters 3 and 4. However, the present study concerning the telomeric length has been carried out with fewer subjects as the second approach to the subjects has largely been unsuccessful and only a few have obliged. Also this aspect has been taken up as a preliminary extension of the major theme of the thesis presented in chapters 3 and 4.

**Average telomeric length determination:**

The telomeric length was determined essentially as described by Kruk et al. (1995).

Briefly the lymphocytes prepared as outlined in chapter 2, were washed with Phosphate buffered saline, lysed in 10mM Tris HCl, 1mM EDTA, 0.5% SDS, 20µg/ml RNase, 0.1mg Proteinase K per ml. DNA was isolated by standard organic extraction as described in chapter 2 and the DNA concentration was determined by absorbence at 260 nm. If the ratio of 260/280 absorbence is less than 1.7 then proteinase K digestion was repeated. The DNA was digested to completion with Hinf I as described in chapter 2. About 3 to 5 µg of restricted DNA was loaded onto 0.5% agarose gel, resolved by electrophoresis at 1V/cm for 26-30 hours in TAE buffer pH 7.5-7.8, transferred to a Hybond N+ nylon membrane and hybridised in Church hybridisation buffer (Church and Gilbert, 1984) with (γ-32P)ATP 5’-end labelled TTAGGG probe. Membranes were washed in 0.5x SSC, 0.1% SDS at 42°C and telomeres were visualised by autoradiography. A typical autoradiogram is shown in Figure 26. Each lane
Figure 26

A typical autoradiogram after Southern transfer and hybridisation using the $^{32}\text{P}-\text{(TTAGGG)}_4$ probe for determination of average telomeric length from lymphocytes of human subjects.
along with standard molecular weight markers was scanned in a computer aided UVP Gel documentation system and the average telomeric length (ATL) is calculated using the formula

\[
ATL = \frac{\sum (MW_i \times OD_i \text{ or Area}_i)}{\sum (OD_i \text{ or Area}_i)}
\]

where OD, or Area, is the desitometric output at position / and MW, is the length of the DNA at position i.

RESULTS AND DISCUSSION

The average telomeric length (ATL) in peripheral lymphocytes of 7 individuals whose age range varied from 46 to 80 years (both sexes) are shown in Table 23 and Figure 27. As can be seen the ATL in these subjects varied from 2.84 Kb to 5.78 Kb. It may be noted that there is a perceptible decrease in the ATL with advancing age, which is in line with the earlier observations of shorter telomeres in older individuals (Hastie et al., 1990; Lindsey et al., 1991; Allsopp et al., 1992; Vaziri et al., 1993).

When six of the seven subjects studied were divided into two groups based on their Body Mass Index (as an indicator of their nutritional status), the low BMI group (BMI less than 19) showed an ATL of 4.96, whereas the Normal BMI group has exhibited an ATL of 3.41 (Table 24). Although this is a pilot study with only a few subjects, there seems to be a general tendency for the LBMI group in the age range studied to possess longer ATL. While more number of cases must be studied before any meaningful conclusion can be drawn, it is nevertheless tempting to speculate that in LBMI group the aging process may have been delayed and therefore the slightly longer ATL may reflect in these cells the potential to undergo more number of divisions as compared to the NBMI group (Harley et al., 1990; Hariey, 1991). However, further studies with an increased sample size are needed to make any affirmative conclusion.
Table 23: AVERAGE TELOMERIC LENGTH IN PERIPHERAL LYMPHOCYTES OF AGING HUMANS SUBJECTS

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>AGE</th>
<th>SEX</th>
<th>BMI</th>
<th>ATL*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>46</td>
<td>M</td>
<td>15.63</td>
<td>4.04</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>F</td>
<td>18.73</td>
<td>5.05</td>
</tr>
<tr>
<td>3.</td>
<td>55</td>
<td>M</td>
<td>24.06</td>
<td>3.41</td>
</tr>
<tr>
<td>4.</td>
<td>55</td>
<td>F</td>
<td>18.43</td>
<td>5.78</td>
</tr>
<tr>
<td>5.</td>
<td>59</td>
<td>M</td>
<td>20.94</td>
<td>3.40</td>
</tr>
<tr>
<td>6.</td>
<td>65</td>
<td>M</td>
<td>19.72</td>
<td>3.41</td>
</tr>
<tr>
<td>7.</td>
<td>80</td>
<td>M</td>
<td>16.96</td>
<td>2.84</td>
</tr>
</tbody>
</table>

* The values are expressed in kilo base-pairs (Kb).
Figure 27

Average telomeric lengths in the peripheral lymphocytes of aging subjects of Indian population. For other details, please see text.
ATL in lymphocytes of aging humans
Table 24: AVERAGE TELOMERIC LENGTH IN PERIPHERAL LYMPHOCYTES
OF LBMI AND NBMI HUMAN SUBJECTS

<table>
<thead>
<tr>
<th>STATUS</th>
<th>AGE</th>
<th>AVG AGE</th>
<th>BMI</th>
<th>AVG BMI</th>
<th>ATL*</th>
<th>AVG ATL*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>46</td>
<td>15.63</td>
<td>4.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>50 ± 5</td>
<td>18</td>
<td>17.60+1.71</td>
<td>5.05</td>
<td>4.96 ± 0.87 #</td>
</tr>
<tr>
<td>3.</td>
<td>55</td>
<td>18.43</td>
<td>5.78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NBMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>55</td>
<td>24.06</td>
<td>3.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>59</td>
<td>60 ± 5</td>
<td>20</td>
<td>21.57+2.24</td>
<td>3.40</td>
<td>3.41 ± 0.01</td>
</tr>
<tr>
<td>3.</td>
<td>65</td>
<td>19.72</td>
<td>3.41</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The values are expressed in kilo base-pairs (Kb).
# This value is significantly higher than the average NBMI value at a p value < 0.05.

LBMI = Low Body Mass Index    NBMI = Normal Body Mass Index
The ATL in two Down syndrome subjects along with age and sex matched controls has been determined and these preliminary observations are recorded in Table 25. The average ATL of the two Down syndrome subjects stands at 2.27 Kb while the same value in control subjects is 3.33 Kb. Thus there is a 1.07 Kb difference in the ATL and this difference could be attributable to the accelerated aging characteristics of Down syndrome subjects. It is already shown that the telomere loss in vivo is equal to 15-50 bp per year (Harley, 1995). Taking the value of 50 bp loss per year, it appears that the DS patients are ahead in aging process by 20 years. It is of course, a pure speculation since only two subjects with the disorder have been studied and much more data are needed to reach conclusions with certainty.

It is however, pertinent to mention that shortened telomeres, as compared to the normals, were noticed in lymphocytes of Down syndrome patients (Vaziri et al., 1993), fibroblasts of Werner's syndrome (Kruk et al., 1995), fibroblasts of Ataxia-telangiectasia subjects (Xia et al., 1996; Metcalfe et al., 1996) and the present preliminary study is in line with the earlier observations.

In the present experiments the ATL found in lymphocytes of normal subjects is somewhat lesser than the values reported by workers from other parts of the world (Kruk et al., 1995; Xia et al., 1996; Slagboom et al., 1996). They reported 5 to 8 Kb whereas in the present study the values are around 4 Kb. The reason for this is not known. One possibility could be the ethnic differences of the population studied in the present investigation.
Table 25: AVERAGE TELOMERIC LENGTH IN PERIPHERAL LYMPHOCYTES OF NORMAL AND DOWN SYNDROME SUBJECTS

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>AGE</th>
<th>SEX</th>
<th>STATUS</th>
<th>ATL*</th>
<th>AVG ATL*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>16</td>
<td>M</td>
<td>Control</td>
<td>4.24</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>M</td>
<td>Control</td>
<td>2.41</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>M</td>
<td>Down Syndrome</td>
<td>2.59</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>11</td>
<td>M</td>
<td>Down Syndrome</td>
<td>1.94</td>
<td></td>
</tr>
</tbody>
</table>

* The values are expressed in kilo base-pairs (Kb).