CHAPTER - V

DISCUSSION
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The total phenomenon of a living human system at any instant is the resultant expression of simultaneous and multiple intricately interrelated metabolic events occurring as essence of the living process either in response to or generating a string of signals to correlate the entire process. The signals responsible for correlating the entire living process is either external to influence the metabolism or internal, generated by the metabolism itself. Physical form of the external signals commonly known as the stimulus from environment are basically different forms of energy which are transduced by the varieties of receptors and receptor systems present at the cellular membranes acting as the dynamic interphase between the self and non self i.e. with the universe outside a metabolic system. In contrast to the signal transduction system from the universe to living system, signal generation and transmission within living systems is mediated by physicochemical processes in the form of biophysical quantum and chemical messengers. In a human living system the fundamental coordinating processes are mediated by concerted activity of the nervous and endocrine systems forming the unified neuroendocrinal axis for pan systematic regulation.

Melatonin is a biomolecule synthesized in the pineal gland (Bowen 2003). A major function of melatonin is to act as the chemical signal for the neuroendocrinal axis related with maintenance of sleeping habits coordinated with periods of darkness and light in the circadian rhythm (Ying-Hui Wu &
Dick F. Swaab, 2004a) which is a factor of considerable importance for a sustained non depressive or normal psychological state (Dean et al, 2000 ). Pineal gland is the only tissue in the human system with the capacity to synthesize and release melatonin with regulatory and modulated periodicity and quantity and responsive coordination between neuropsychological metabolic need and exposure to quantum of electromagnetic radiation in the visible range. The uniqueness of pineal gland as the source of melatonin under modulation of phototropic signals through the retino-hypothalamic tract and the relationship between the trend and degree of fluctuations of melatonin availability to the neuroendocrinal metabolism related with maintenance of a psychological state without depression logically inspires for undertaking the present study to probe the relationship among pineal gland, melatonin and ‘severe depressive episode’.

In the present study circulating melatonin level in the form of serum melatonin is estimated as an index for functional status of the pineal gland in subjects broadly grouped as reference control consisting of normal healthy individuals without any depressive symptoms and experimental variable formed by persons with ‘severe depressive episode’ as screened by the ICD-10 Classification of Mental and Behavioral Disorders, clinical descriptions and diagnostic guidelines, World Health Organization Geneva 2002.

Relationship between functional activity of pineal gland and non depressive / depressive states in the subjects are analyzed under matched sets of observation using the null hypothesis: Reject $H_0$ if $P < t_\alpha$ when $t_\alpha = t_{0.05}$ setting the level of confidence at 95% probability signifying that if the differences in observation between the matched groups is significant at the level of $P < 0.05$ (111)
the hypothesis will be rejected establishing differences in pineal functions between the tested groups.

To probe the structural integrity and dimensional differences in the pineal glands of the subjects under the current investigation, radiological probing through application of M.R.I. has been adopted and the observation is put under the same null hypothesis set as criteria for rejection. To probe the relationship between structural and functional components of the pineal gland and few other relevant variables as age and sex of subjects, the observations are put under correlation analysis.

**MELATONIN:**

Considering the differential trends in melatonin secretion by pineal gland under the circadian rhythm and variations in exposure to darkness and light as reported by various workers on the allied subject (Brownstein and Heller, 1968), (Law, 1986), (Ying-Hui Wu & Dick F. Swaab, 2004a) in the present investigation the samples for evaluation of pineal function through serum melatonin estimation has been collected between midday and afternoon which is extensively reported (Dean *et al*, 2000) as the period of generalized depletion for circulating melatonin levels. The time adopted for collection of samples is expected to buffer the fluctuations in circadian melatonin alterations and represent the residual melatonin in circulation and thereby acting as the baseline index for the 24 (twenty four) hour cyclic variations.

The distribution pattern of the number of subjects among different age groups in both control and patients with ‘severe depressive episode’ is
symmetrical without any appreciable skewness signifying homogenous composition in both the groups and thereby establishing proper age matched experimental conditions for the present study. The distribution pattern is presented in fig. 4.

In the present study the mean serum melatonin level under the adopted experimental setup is found to be elevated with very high significance (P<0.001) in patients with 'severe depressive episode' (106.52±19.1 pg/ml) than the control group(6.932±0.278 pg/ml). As evident from the fig. no. 2. presenting the pattern of distribution of serum melatonin in the two groups it is easily observed that in the subjects with 'severe depressive episode' the serum melatonin concentration is almost uniformly distributed towards the higher side of the scale with only a few observation in the portion of the scale occupied by major area covered by the normal control group signifying (P<0.001) presence of only a few isolated cases of severe depressive episode corresponding with the normal range of serum melatonin under the present experimental setup. In contrast to the trend of serum melatonin in 'severe depressive episode', the distribution pattern in the normal control group is presented with a distinct biphasic character signifying presence of two subgroups (P<0.001) under the control population of the present study. It is definite from the observed trend that, the mean serum melatonin in both the subgroups of the control population is significantly low (P<0.001) than the patient with 'severe depressive episode'; but there is a definite fraction of normal population with serum melatonin levels nearing the origin of peak serum melatonin under 'severe depressive episode'. The serum melatonin level in patients with 'severe depressive episode' is observed to be apparently highest
with a mean value of 174.82±37.769 pg/ml in the age group of 30 to 39 years without any significant differences (P>0.05) with the mean melatonin levels of the other age groups in this group of patients with 'severe depressive episode' as presented in figure no.2. and table no. 13. However, among the different age groups, the mean serum melatonin level does not show any fluctuations (P>0.05).

The correlation analysis to study the probability of any relationship between age and serum melatonin level in both control and the cases with 'severe depressive episode' does not show any persistent relationship among these parameters in both the groups under the present study with coefficient of correlation R=0.093 and R=0.031 respectively for control and cases as presented in fig. no. 10.
Co-relation between age and melatonin

\[ y = -0.4947x + 40.702 \]
\[ R^2 = 0.0088 \]
\[ R = 0.093 \]

\[ y = -0.0029x + 31.892 \]
\[ R^2 = 0.001 \]
\[ R = 0.031 \]

Figure no. 10: Figure showing relationship between age and serum melatonin in both the groups
PINEAL GLAND:

Under the present study the gross anatomy of the pineal gland have been studied utilizing radiological techniques with application of M.R.I. and giving emphasis on the location and dimensional aspects of the pineal gland in 50 (fifty) subjects. With the procedure and protocol adopted for location and probing the dimensions of pineal gland through M.R.I. under the present experimental set up, the pineal gland could not be located in 6 (six) subjects and in all remaining subjects in this group the pineal gland is located in its normal expected anatomical position. With the present experimental setup the observed inability to locate the pineal glands in the few subjects and the factors associated with this could not be ascertained definitely under the scope of the present study.

The mean length presenting the antero-posterior diameter of the pineal gland in the group of subjects in the present study if observed as $4.606 \pm 2.3\text{mm}$ inclusive of all age groups and both sexes consisting the study group. The range of length of the pineal gland is from 2.1 to 9 mm excluding the subjects where the pineal gland could not be located. The distribution pattern of the pineal gland length is skewed to the right indicating presence of a group of subjects with longer pineal glands of more than 6.00 mm having a relative frequency of 0.436 covering the ranges from 6 mm to 10 mm depicting that about 50% of the subjects under study have pineal gland more than 6 mm in its longitudinal axis.

The dimensions of the pineal gland at right angle to the longitudinal axis indicating the breadth of the pineal gland under the present setup of radiological probing reveals that the mean breadth presenting the cranio-caudal
diameter in the group of subject is 2.716±0.202 mm with a range of 1.4 mm to 7.4 mm excluding the subjects where it cannot be located. The distribution pattern of pineal gland breadth is normal without any apparent skewness suggesting that the distribution of pineal gland breadth is more consistent than its length.

In the present study the mean length of the pineal gland for the male subjects is found to be 4.725±0.463 mm and in the female subjects the mean length is 4.465±0.476 mm. The mean breadth of pineal gland for male and female subjects is found to be 2.740±0.272 mm and 2.686±0.341 mm respectively. No significant difference (P>0.05) is observed in both the dimensions of pineal gland between male and female subjects signifying absence of any appreciable differences in the size of the pineal gland between male and female. As presented in figure no. 9, the distribution pattern of length and breadth of pineal gland in male and female subjects also does not show any appreciable differences and is almost identical supporting the fact that size of pineal gland is not significantly different between male and female.

Under the present setup for anatomical study of the pineal gland by radiological technique with M.R.I. evaluation of dimensions of pineal gland measured in saggital section the coefficient of correlation between pineal gland length and breadth in the group of subjects under the present investigation is observed to be fairly high with R=0.692 signifying a persistent relationship between the two dimensions (Fig. 11). On differential correlation of the same parameters of pineal gland length and breadth in male and female subjects of this group it is observed that similar levels of correlation exists in both male
Correlation of length and breadth of pineal gland

\[ y = 0.4065x + 0.8437 \]

\[ R^2 = 0.3964 \]

\[ R = 0.692 \]

Figure 11: Correlation between length and breadth of pineal gland for whole group
The relation between length and breadth of male and female Jiu; mpeajg is shown in Figure 12.

For males:

- Linear equation: \( y = 0.452x + 0.6687 \)
- Coefficient of determination: \( R^2 = 0.3975 \)
- Correlation coefficient: \( R = 0.630 \)

For females:

- Linear equation: \( y = 0.3731x + 0.9776 \)
- Coefficient of determination: \( R^2 = 0.4031 \)
- Correlation coefficient: \( R = 0.634 \)

Figure 12: Comparison of correlation between length and breadth in male and female pineal glands
Figure 13 Correlation between age and length of pineal gland in the age group of below 20 years.
Figure 14: Correlation between age and length of pineal gland in age group of below 20 to 40 years

\[ y = 0.1079x + 2.7295 \]
\[ R^2 = 0.0962 \]
\[ R = 0.310 \]
Figure 15: Correlation between age and length of pineal gland in age group of 40 to 60 years

\[ y = 0.0649x + 1.6872 \]

\[ R^2 = 0.1103 \]

\[ R = 0.332 \]
Figure 16: Correlation between age and length of pineal gland in age group of 60 years and above.
and female subjects with R=0.634 and R=0.630 respectively indicating uniformity in the relationship of pineal antero-posterior and cranio-caudal diameter in both the sexes (Fig. 12).

On analysis of the age related dimension of the pineal gland as presented with the scatter in figure. 13, it is initially observed that apparently there is no any dependency between the age and size of the pineal gland in the subjects under present investigation as evident from the correlation coefficients of age with length and breadth with R=0.21 and R=0.15 respectively when considered as a single group formed by the total number of subjects spread over the range of age from 1(one) year 3 (three) months to 85 (eighty-five) years. The present observation on the total series covering the wide range of age span may prompt to interpret that pineal gland size is independent of age of the subjects and there is a fairly constant relationship between the antero-posterior and cranio-caudal diameter making the pineal gland an ovoid body in both the sexes. But considering the well established fact that the universally followed path for organogenesis during development, maturity and sustenance of any system, each organ gas a predefined span in life cycle for attainment of peak maturity followed by some degree of regression within limits of distribution accepted as normal; at this stage of the present discussion a fractionated correlation analysis is applied over the parameters under consideration on the same group of subjects by sub-grouping them on basis of age as ‘below 20’, ‘20 to below 40’, ‘40 to below 60’, and ‘60 and above’. From the fractionated correlation performed over the different age groups as presented in fig. 14, 15, 16, and 17 it may be clearly interpreted that there is a very high degree of correlation with R=0.762 between
age and antero-posterior length of pineal gland in the age group of 'below 20' years. In the succeeding slots of age with age between '20 to below 40' and '40 to below 60' the coefficient or correlations between the studied parameters are R=0.312 and R=0.332 respectively followed by the lowest degree of correlation with R=0.014 in the age group of 'above 60' years of age. The presented trend of relationship between age and pineal gland length in different age groups under the current investigation clearly depicts that the size of the pineal gland (length) increases proportionately upto the age of 20(twenty) years followed by a period of constancy in length independent of increase in age as evident from the lowered and almost identical correlation coefficient during this period of age. The observed reduction of correlation between pineal gland size(length) and age almost to the nil with a value of only R=0.014 clearly establishes that there is some degree of regression in size along with advancement of age without any relationship with the increasing age.

In this present state of discussion over the relationship between age and pineal gland dimensions the observation and interpretation presented on the basis of correlation with fractionated age groups is further justified with a set of comparison of mean pineal gland length in different of at age groups under the present experimental setup. On comparison of the mean pineal gland size (length) in the age group of ‘20 to below 40’ with ‘below 20’ and the other two slots covering from 40 onwards it has been observed that the mean pineal length is significantly high (P<0.05) than the remaining three groups. It may be noted here that in the present study the lowest mean pineal gland length (3.45±0.702 mm) is observed in the age group of ‘below 20’ years and the maximum mean
pineal gland length (5.715±0.651 mm) is observed in the age group of '20 to below 40'. With reference to the fig.no.5(a) present the trend of mean pineal gland dimensions in different age groups there is a significant (P<0.05) burst of increase in pineal gland size between 20 to 40 years of age followed by any insignificant (P>0.05) changes with ages over 40 years. The concerted analysis over the relationship between age and size of pineal gland with fractionated correlation studies under the present setup with M.R.I. adopted radiological probing of the pineal gland for assessment of pineal dimensions and its trend of changes with age it may be finally commented that the maximum pineal dimension upto the age of 40 characterized by a phase of augmented growth during the period of adolescence and youth followed by a period of sustained size ultimately leading to the final phase of insignificant (P>0.05) regression after crossing the age of 40 years emphasized with the fact that the augmented growth phase is age dependent but the sustained and regressive phase are age independent as far as their rate of change in size is considered.

FINAL DISCUSSION:

In the present study over pineal gland, melatonin and 'severe depressive episode' the serum melatonin as estimated under the already discussed experimental setup represents the baseline melatonin index in the daytime samples during its diurnal variations expected to represent the residual circulating melatonin after the nocturnal fluctuations in both group of subjects with or without 'severe depressive episode'. Mean serum melatonin concentration in subjects with 'severe depressive episode' is found to be significantly high than (126)
the control group of normal subjects which may appear as contradicting with reports of related works on serum melatonin concentration under different conditions, situations and experimental setup (Thompson et al, 1988), (Webb and Puig-Domingo, 1995), (deVries et al, 1997), (Dolberg et al, 1998), (Dalton et al, 2000), (Kripke et al, 2003), (American Accreditation HealthCare Commission, 2008). In most of these studies over serum melatonin concentrations with or without depressive episodes, the nocturnal serum melatonin status was reported which may be a factor for the discrepancy with the findings of the present study.

However, with reference to the well established ‘serotonin hypothesis’ (Gibbons RD and Davis JM, 1986) it may be tried to explain here that in presence of conditions associated with ‘severe depressive episode’ if somehow the biosynthesis of melatonin is hampered then there will be increase in melatonin level even at day time without neuronal stimulus of darkness resulting in increase in day time basal melatonin in circulation as observed in the present study. One of the contrasting trends in serum melatonin level is in the distribution pattern between control and the group with ‘severe depressive episode’. In the ‘severe depressive episode’ group the serum melatonin is found to be fluctuating under different age groups but in contrast to this there is no any fluctuation in the serum melatonin level in the control group. The differences in the observed trends may be the consequences of different degree of metabolic alterations at different ages in patients with ‘severe depressive episode’ where alterations in melatonin metabolism may be a primary factor for the condition.

(127)
which is logically expected to be absent in the control group explaining the non fluctuating behaviour of serum melatonin in different ages. In relation to the dimensions of the pineal gland probed under the present experimental setup it is clearly established by the observation and analytical interpretation that the peak pineal size is attained during adolescence and youth with insignificant regression with ages over 40 years. On a concerted interpretation of the observation and analyzed outcome of the present study on pineal gland, melatonin and ‘severe depressive episode’ it may be finally opined that day time serum melatonin level is significantly elevated in subjects with ‘severe depressive episode’ and size of the pineal gland is related with age, only upto 40 years following which there is no any age related dependency of pineal size studied under the limitations of the presented setup.