CHAPTER 4

METHODS

This chapter explains the various materials and methods used to fulfill the aim and objectives laid down for the research.

4.1 INTRODUCTION

The research included two studies conducted at two independent HIV/AIDS rehabilitation centers(RCs) involving children/adolescents. Before selecting the RCs, several RCs were visited to find out whether it would be possible to conduct the study in the centers. Based on the availability of the subjects and acceptance by the RCs, the RCs were selected. The overall aim and objectives of the two studies were the same. However, the study variables were slightly different. The first study was conducted at M/s Snehadān, a rehabilitation center (RC1) in Bengaluru, Karnataka, India. The center had 94 children/adolescents in the age group of 5 to 19 years, including both boys and girls. The second study was conducted at M/s Amma mane a rehabilitation center (RC2) in Mysuru, Karnataka, India. The center had 22 children in the age group of 6 to 18 including both boys and girls. The details of the two studies are explained separately in the following sections. Table 4.1 gives an overview of the components of the two studies.

Table 4.1: Overview of the components of the current research

Design descriptors/variables	**Study ONE @Snehadān (RC1)**		**Study TWO @Amma mane (RC2)**	
Design of Study	Two groups (Yoga & Control) RCT Study		Single group Pre-Post study	
Time	Pre	Post	Pre	Post
No. of subjects	73	69	22	18
Socio Demographic Data	☑	☑	☑	☑
IMMUNE PARAMETERS				
CD4 cell counts	☑	☑	☑	☑

Design descriptors/variables	Study ONE @Snehadān (RC1)		Study TWO @Amma mane (RC2)	
CD4/CD8 ratio	☑	☑	☑	☑
Viral load	-	-	☑	☑
PSYCHOSOCIAL PARAMETERS				
PedsQL ™ Health Related Quality of Life	☑	☑	☑	☑
PedsQL ™ Fatigue Related Quality of Life	☑	☑	☑	☑
Children's Depression Inventory	☑	☑	☑	☑
COGNITIVE FUNCTIONS TESTS				
Six Letter Cancellation Test	-	-	☑	☑
Digit Span Forward Backward test	☑	☑	☑	☑
Stroop Word and Colour test	☑	☑	☑	☑
Symbol Digit Modalities Test	☑	☑	☑	☑

4.2 STUDY ONE

The details of the study at Rehabilitation Center-1 (RC1) is given below.

4.2.1 Design of the study

The design of the study was a randomized control trial. More details of the study are as follows.

4.2.1.1 Source of Subjects

Subjects for the study were from "Snehadān", a rehabilitation center for HIV positive children/adolescents, located at Ambedkar Layout, Carmelaram Post, Sarjapur Road, Bangalore.

4.2.1.2 Inclusion criteria

The inclusion criteria are as follows:

- Subjects with documented HIV+ status
- Able to read, write, understand English
- Age group between 6-19 years
- Both male and female subjects
- On regular treatment like the ART or other treatment, or not on any treatment

4.2.1.3 Exclusion Criteria

The exclusion criteria are listed as follows:

- CD4+ T-cell count < 200 cells/μL of blood
- Subjects with serious/severe comorbid psychiatric illnesses
- Subjects with other major comorbid illness viz., Fungal Infection in the last 1 month, cancer, Tuberculosis.

4.2.2 Screening and recruitment

Initially, 94 children of RC1 were screened. 16 were excluded on the basis of age, leaving 78 children. Out of 78 children, five were excluded based on co-morbid illness criterion due to tuberculosis (Figure 4.1). However, yoga was given to all those who were in the yoga group; but were considered as dummy candidates and excluded for data analysis. Four candidates, three from control group and one from yoga group dropped out of the study since they left the rehabilitation center. Four candidates left the rehabilitation center, and hence were dropouts from the study.

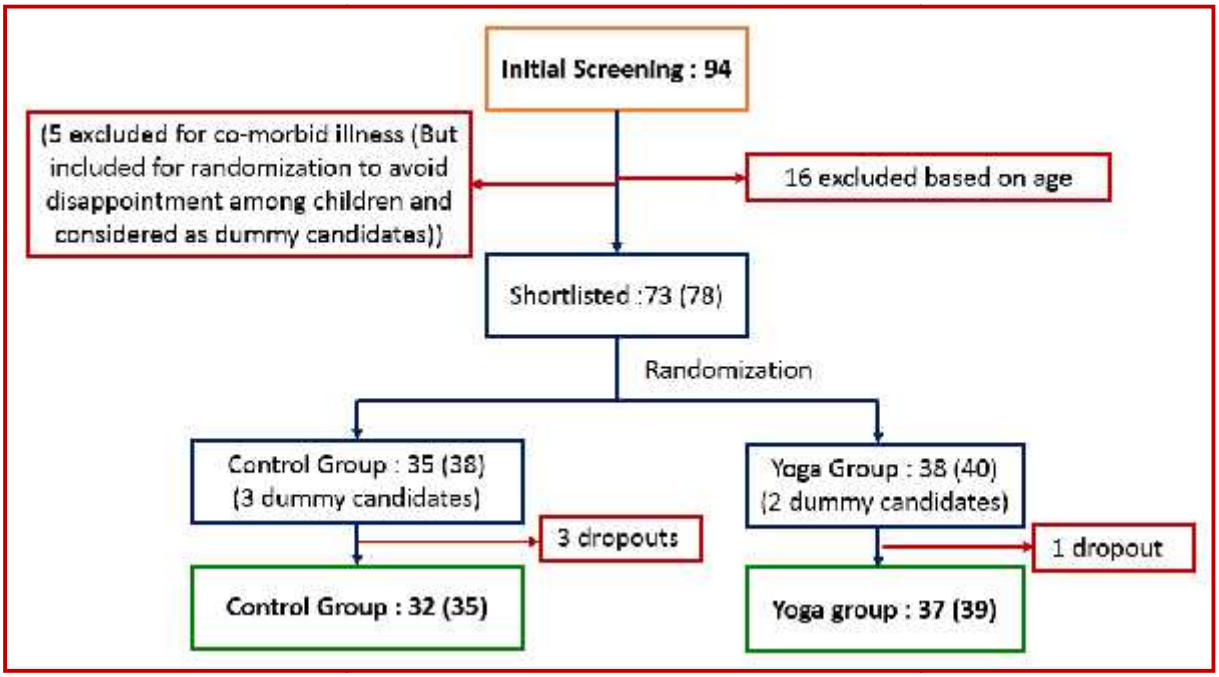

Figure 4.1: Consort diagram

4.2.2.1 Sample size

The sample size was calculated based on a reported pilot study(Creswell *et al.*, 2009). The sample size was calculated using GPower 3.1 software. As per the calculations, consideringthe level of significance, $\Im = 0.05$& the power,$1-\wp = 0.80$ a sample size of 38 was suggested (Appendix II). Out of 94 children/adolescents in RC1, 73 satisfied the inclusion criteria. Among this some were on ART (40%) and some not (60%), making it difficult to find equal representation for on-ART and not-on-ART individuals in the intervention and control groups. Hence it was decided to include all the 73 in the study.

4.2.3 Randomization

The participants were to be divided into two groups, Yoga Group (YG) and Control Group (CG). A process called minimization was used for randomization(Taves, 1974; Scott *et al.*, 2002). In this process, the subjects are arranged in an order (ascending/descending) based on one or more parameters in the research; and are then, in the order randomly assigned to the different groups. This would help reduce differences in the parameters at baseline between groups.

In the current research, the socio-demographic data of the subjects were entered in Excel under the headings, name, participant number, gender, age, ART status and CD4 cell count. The subjects were sorted based on gender, ART status, Age and CD4 cell count. Sort function in Microsoft Excel was used for the purpose. (Figure 4.2)

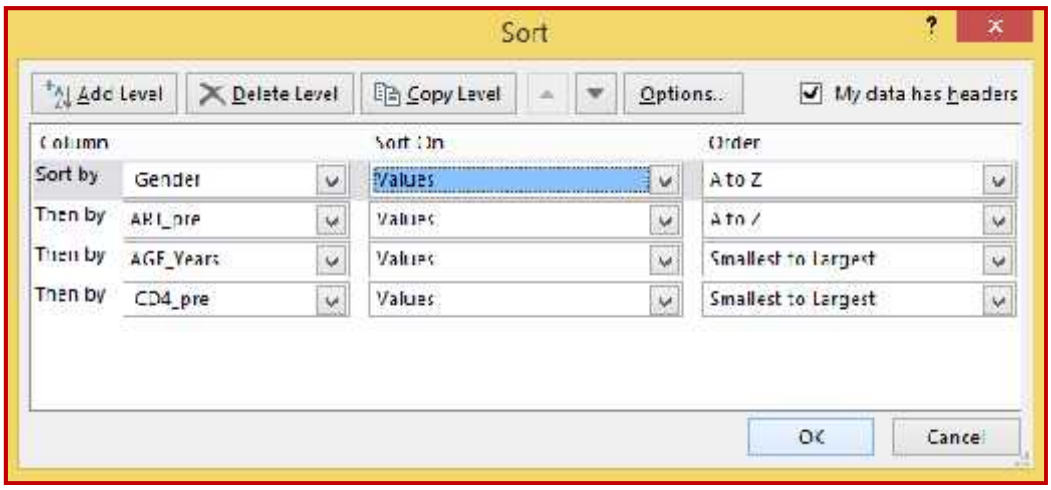

Figure 4.2: Sort function in Microsoft Excel

The sorted list of subjects thus prepared was used for randomization. Two chits with numbers 'one' and 'two' were prepared. The subjects were called in order from the list of names prepared, two at a time. The two of the subjects were asked to pick a chit out of the two chits. If the number in the chit they picked was 'one' they would belong to group one, and if it was 'two' they would go to group 'two'. The same was done with all the subjects in groups of two. At this stage, the subjects were only divided randomly into two groups; while none of them knew whether they would be in YG or in CG. Once all the subjects were divided into two groups, anyone participant from each group was asked to volunteer to come ahead. The decision as to whether the group they represented would be in YG or CG was based on tossing of a coin. It was instructed before tossing that the toss winning group would go to YG. Thus Group-2 which won the toss went to YG.

4.2.4 Ethics clearance and consent

Informed consent form, part of the ethics clearance, for all the participants was signed by the director of Snehadān as the legal guardian of the subjects. Once the design of the study was through, ethics clearance was sought from the 'Institutional Ethics Committee' of S-VYASA University, Jigani, Bangalore. The design was presented to the panel of the IEC members. The IEC after discussion gave clearance for the study. The consent provided by the Director for the study is given inAppendix III (INFORMED CONSENT FORM).

4.2.5 Intervention

Yoga intervention mainly included an integrated approach using multidimensional techniques for the strengthening of the body, mind and immune system. The intervention was largely based on a potential yoga module for HIV positive individuals(Bhargav *et al.*, 2010). The intervention was for 4 months. It had two parts:

1) 50-55 minutes of yogic practices including warm-up, āsana, and prāṇāyāma practice; 5 days a week held early in the morning, between 6-7 am
2) 50-55 minutes of yogic games per day; 5 days a week held between 3-4 pm.

During this period, the participants who were part of the control group had their routine sleep during the morning hour and played football or other outdoor games during the 3-4 pm session; both of which were part of their daily routine. Routine medical care too was not disturbed.

This research also was an attempt to study the effect of IAYT on HIV seropositive individuals, the participants being largely children, and also due to time constraints, all components of IAYT could not be included. The detailed components of the intervention are indicated in Table 4.2.

Table 4.2: Components of yoga intervention (RC1)

Sl. No.	Intervention component	Approx. time for practice	Schedule
1	Loosening exercises and breathing exercises	8-10 minutes	Daily*
2	Kapālabāti	~3 minutes	Daily
3	Surya namaskār (3-6 rounds)	8-10 minutes	Daily
4a	Other āsanās: ardhakaṭi cakrāsana, ardha cakrāsana, uṣṭrāsana, paścimottānāsana, śalabāsana	8-10 minutes	Randomly during first four months
4b	Other āsanās: vṛkkṣāsana, gomukhāsana, supta vajrāsana, pārśvakonāsana, ardhamatsyendrāsana, śalabāsana, dhanurāsana	8-10 minutes	Randomly during second four months including ones in 4a

Sl. No.	Intervention component	Approx. time for practice	Schedule
5	Padmāsana		During prāṇāyāma
6	Vajrāsana	~2 minutes	Emphasized while giving general instructions
7	Nādi shodhana prāṇāyāma, Vibhāgiya Prāṇāyāma	5-8 minutes	Daily
8	Bhramari, Ujjāyi Prāṇāyāma, Bastrika Prāṇāyāma, mārjala kriya	5-8 minutes	Randomly
9	prāṇāyāma: Nādi shodhana prāṇāyāma, Vibhāgiya Prāṇāyāma, Bhramari, Ujjāyi Prāṇāyāma, Bastrika Prāṇāyāma	8-10 minutes	Daily, at random
10	QRT, Shavāsana/DRT	5-8 minutes	Daily
11	Yogic games	~50-55 minutes	Daily

* Five days a week

Yoga was taught as per the recommendations given in "Yoga for Promotion of Positive Health"(Nagarathna and Nagendra, 2011). Similarly, the yogic games included random games used to improve cognitive functions as recommended in the text 'krida yoga' (Dattaram Pol, 2015).

4.2.6 Data collection

Data collection was at two-time points. The first set of data was collected at the beginning of the study. The second set of data at the end of the intervention. Although data were collected from all the participants, due to loss during transit some data pertaining to the questionnaire and cognitive functions got lost. Hence the number of participants in some of the variables is lesser than the total participants.

4.2.7 Registration of the study

The study was retrospectively registered with the Clinical Trial Registry of India. The registration number of the trial is CTRI/2018/05/014030

4.3 STUDY TWO

The details of the study at Rehabilitation Center-2 (RC2) is given below.

4.3.1 Design of the study

The study was a single group one time pre-post study.

4.3.1.1 Source of subjects

The subjects were the children/adolescents (C/As) from “Amma mane”, a rehabilitation center for HIV positive individuals.

4.3.1.2 Inclusion criteria

All children and adolescents in the Rehabilitation Center (Age group 8-18) were included in the study.

4.3.1.3 Exclusion criteria

Although all children and adolescents were included in the study, in case of tests requiring knowledge of reading, writing and understanding English, only such C/As capable of the same were considered.

4.3.1.4 Sample size

All the 22 subjects in the rehabilitation center were considered for the study. However, due to constraints, mentioned in the exclusion criteria, some children/adolescents were excluded from data collection.

4.3.2 Intervention

The practices in the yoga intervention mainly included all the practices explained in study one except for the yogic games. In addition, the study included practice for the spiritual aspect, like ‘om’ chanting which could not be included in study one. Further, the total

practice time was one hour, seven days week and for six months. The daily routine of the subjects was not disturbed, except for the one-hour yoga program. Further, the routine medical care too was not disturbed; all the participants continued the standard medical care and check-ups as per general norms. All the participants were on ART routine as per part of the standard medical care.

4.3.3 Data collection

Data wascollected at two time points; the first set at the beginning of the study and the second set of data at the end of six months of intervention.

4.4 DATA CLEANSING AND ANALYSIS

With reference to the questionnaire, in cases where the C/As had given multiple responses or no responses, the responses were imputed as per the instruction manual of the questionnaire developers. When a participant was not able to or did not want to answer a questionnaire, or in cases of loss of data, the data was not imputed.

In study one, the number of participants being >30 normal distribution was assumed. In study two, the number of participants being 22, the data was tested for normality using the Shapiro-Wilk test. Since the distribution was not normal, non-parametric tests were conducted. T-test (Paired sample T-test (to test within-group differences) and independent sample T-test (to test between-group differences)) and Wilcoxon signed-rank test (WSRT) were used for determining the significance of change between pre and post results in the parameters for data with normal distribution and with non-normal distribution respectively. To determine the group * time interaction effect (study 1); i.e. along with group effect and time effect whether there is any significant interaction between group and time, which means, as both group and time change, whether there is a significant difference in the parameter considered, analysis of variance (ANOVA) was performed at 0.05 level of significance.

Data entry was done using Excel 2013. Data analysis was carried out by writing appropriate codes using the statistical programming language R, versions 3.3.3 to 3.5.2. Typical R codes developed are shown in Appendix IX.

4.5 OUTCOME MEASURES

The outcome measures considered in the current research are:

- General health status
- Socio-demographic data
- CD4, CD4/CD8 ratio, viral load
- Quality of life Assessments through PedSQL questionnaire
- Depression using the Children's Depression Inventory ver.2 full length (CDI2-SR) questionnaire
- Cognitive functions:
    - Stroop Test (ST)
    - Digit Span Forward Backward Test (DFSB)
    - Symbol Digit Modalities Test (SDMT)
    - Six Letter Cancellation Test (SLCT)

Due to practical limitations, some outcome measures were either not available or not able to be facilitated in the RCs. The results of the outcome measures are reported in the results chapter (Chapter 5) considering the limitations. The details of these measures are given in the following sub-sections.

4.5.1 Socio-demographic data

The age/date of birth was collected from the files maintained by the rehabilitation centers (RCs).

4.5.2 Anthropomorphic variables

The height (m) and weight (kg) of the participants was collected from the medical records maintained on a regular basis as per standard protocol. BMI was calculated using the formula, BMI = Weight / Height^2.

4.5.3 General health

General health status was collected from the records as reported by the physicians during the routine checkup, maintained at the rehabilitation centers.

4.5.4 Immune responses

The immune responses included CD4 cell counts, CD4/CD8 ratio and viral loads. CD4 cell counts were collected from the files maintained on standard, routine basis by the RCs. As reported, the blood samples were analyzed using the FACSCalibur^{TM} flow cytometer. CD8 cell counts were not available from the files. However, CD3 cell counts were available using which CD8 cell counts were calculated using the formula; CD8 cell counts = CD3 cell counts – CD4 cell counts. From the CD8 cell counts thus obtained, the CD4/CD8 ratio was calculated. Viral load was also collected from the files, as available. However, the blood test conducted to determine the viral load in the case of RC1 was limited to a negligible number of candidates. Hence viral load is not included in outcome measures of study at RC1. Even in RC2, the blood test reports were available for only limited participants. Hence for immune response data of only 11 participants were available and the same is reported here.

4.5.5 Quality of Life

The health-related quality of life (HRQOL) of the participants was assessed using the PedsQL^{TM}4.0 Quality of Life questionnaire. The fatigue related quality of life (FRQOL) was assessed using the PedsQL4.0 fatigue questionnaire. The questionnaires are shown in

Appendix VI.1. The questionnaires are answered based on a 5 point Likert scale, the options and scoring for the same is as follows: 0-Never, 1-Almost never, 2- Sometimes, 3-Often and 4-Almost always. The respondent chooses one of the five responses based on their past one month experience. The questionnaire, developed by M/s Mapi Research Trust, is designed for different age groups; of which the one relevant to the current research is the age group of 5-7, 8-12, 13-18. Two participants in RC1 were of 7.58 and 7.77 years at baseline. They were also administered a questionnaire of group 8-12 years. The questions in the questionnaire of groups 8-12 and 13-18 were the same, except in addressing their peers as kids and teens respectively. Hence although the questionnaire was administered as per the age, for analysis they could be clubbed together. The HRQOL questionnaire has four sub-scales. The FRQOL questionnaire has three sub-scales. A brief overview of the HRQOL and FRQOL scales and subscales are shown in Figure 4.3 and Figure 4.4 respectively.

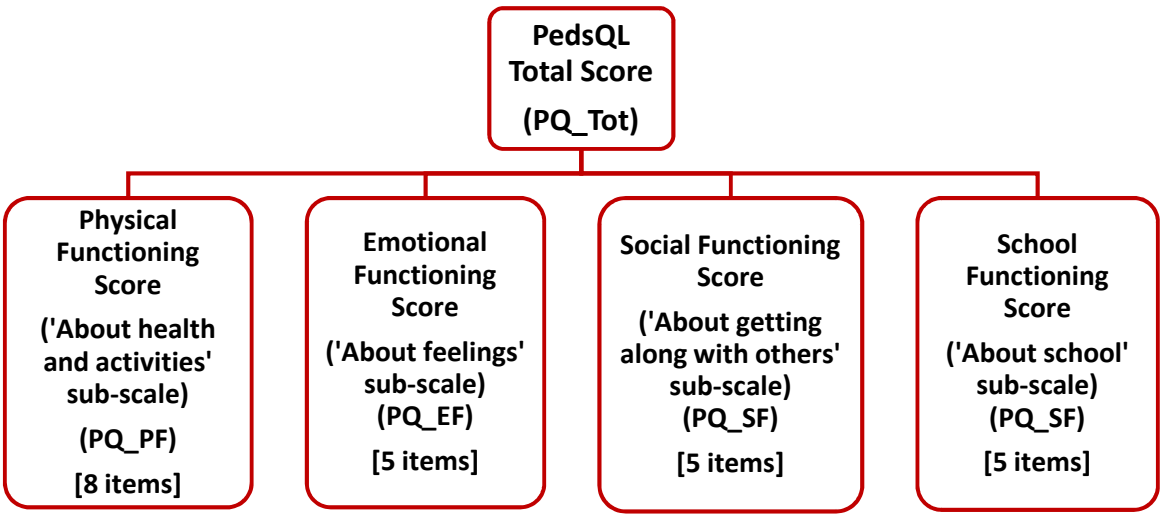

Figure 4.3: Details of PedsQL QOL sub-scales

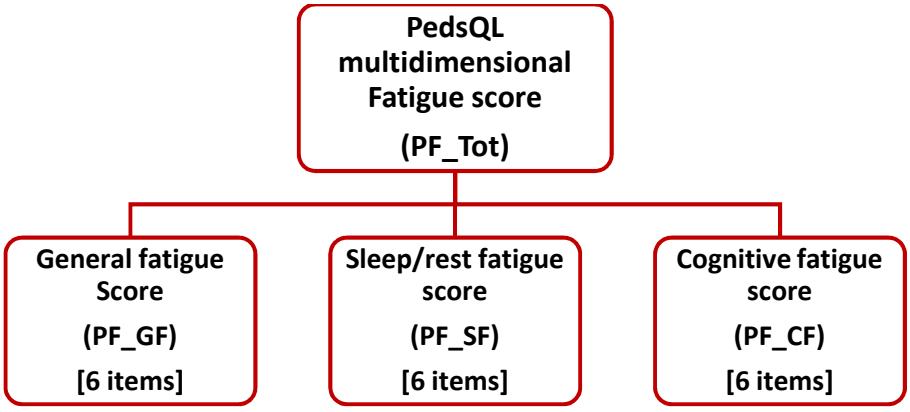

Figure 4.4: Details of PedsQL Fatigue sub-scales

4.5.6 Depression

The current research uses the Children's Depression Inventory Version 2 (CDI 2^{TM}) questionnaire developed by Maria Kovacs of Multi-Health Systems Inc. for assessing depressive symptoms of the participants. In particular, the self-reported full-length questionnaire (CDI 2: SR) was used. CDI 2: SR is a 28 item Likert based questionnaire with three answers out of which the child is supposed to tick one of them depending on the status of the child in the past 15 days. The three answers (options) scored; in order, as 0, 1 and 2 indicate absence, mild presence and a severe indication of symptom respectively. Figure 4.5 shows the components of the interpretation of the CDI scores. The total score is split into two components, viz., emotional problems score and functional problem score. These are further subdivided into a total of four subscales viz., 1. Negative moods/physical symptoms, 2.Negative self-esteem, 3.Interpersonal problems and 4.Ineffectiveness (Figure 4.5). The computations are made as per the CDI-2 manual (Kovacs, 2012)published by the questionnaire developer.

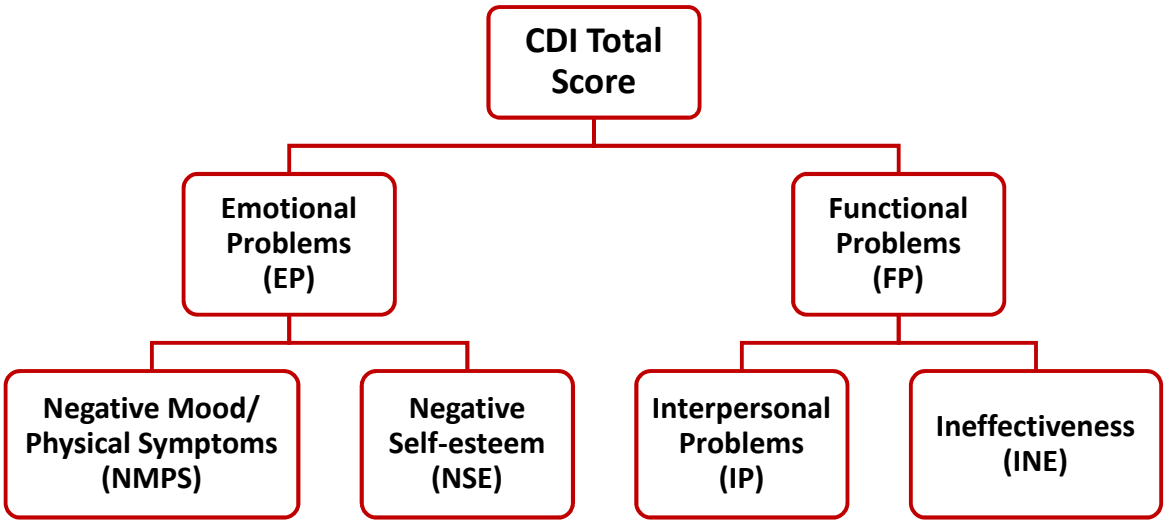

Figure 4.5: Components of CDI score

Based on the age, the T-scores were noted from the raw scores for total and subscale scores using the CDI2-SR user manual. Based on the T-scores, the CDI2-SR classifies depression status into four categories, 1) Very elevated, 2) Elevated, 3) High average and 4) Average or 5) low(Kovacs, 2012)(Figure 4.6).

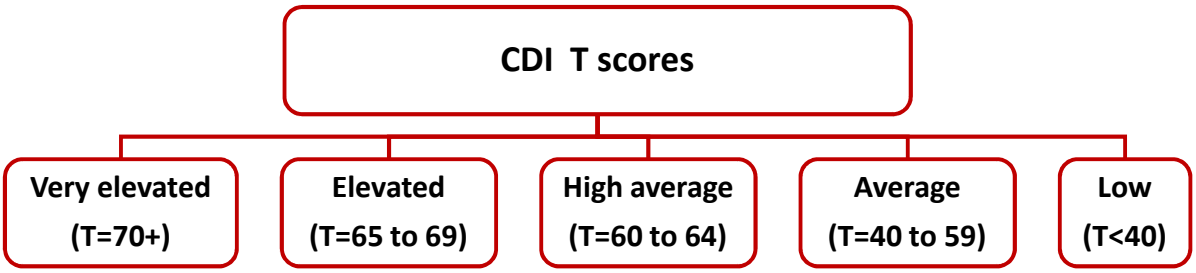

Figure 4.6: Categorization of CDI according to CDI2-SR

4.5.7 Cognitive functions tests

Cognitive Functions (CFs) were assessed through Digit Span Forward Backward (DSFB) test, Symbol Digit Modalities Test (SDMT), Six Letter Cancellation Test (SLCT) and Stroop Test (ST). While DSFB and Stroop Tests test the psychomotor performance (PP)(Purohit and Pradhan, 2017), SLCT and SDMT test the executive functioning (EF) aspect of CF. Standard test procedures were used for assessment in all tests.Figure 4.7 shows the cognitive function

tests used in the current research. The details of the assessments are explained in the following sub-sections.

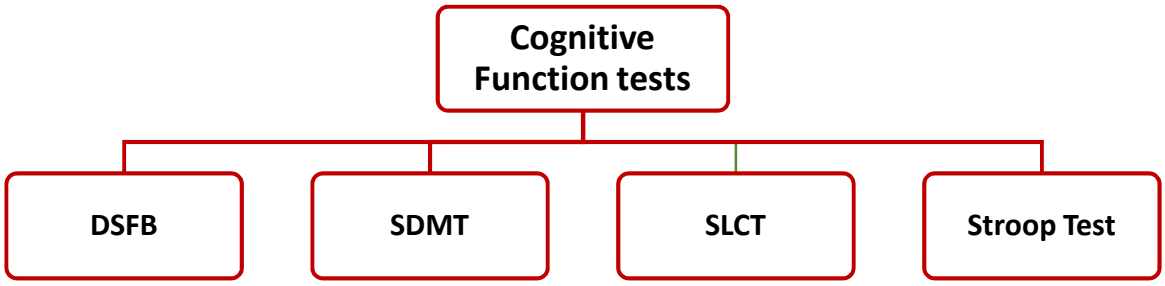

Figure 4.7: Cognitive Function tests

4.5.7.1 The Six Letter Cancellation Task (SLCT):

SLCT is a very commonly used tool for determining the cognitive functions of children and geriatrics.SLCT is used to measure CFs such as concentration, selective attention, focused attention(Jagannathan, Raghuram and Talwadkar, 2014). In particular, the task is used to measure psychomotor performance. The test sheet consists of a matrix of English capital alphabets in 22 rows and 14 columns which hassix target letters, also randomly scattered in the matrix of letters. A sample SLCT form is shown in Appendix VI.2.

The participant is required to strike out as many target letters as possible in 90 seconds. SLCT score has three components, each measuring different brain functions (Rueckert and Grafman, 1996; Bhuyan and Mishra, 2013). Figure 4.8 shows the significance of these components.

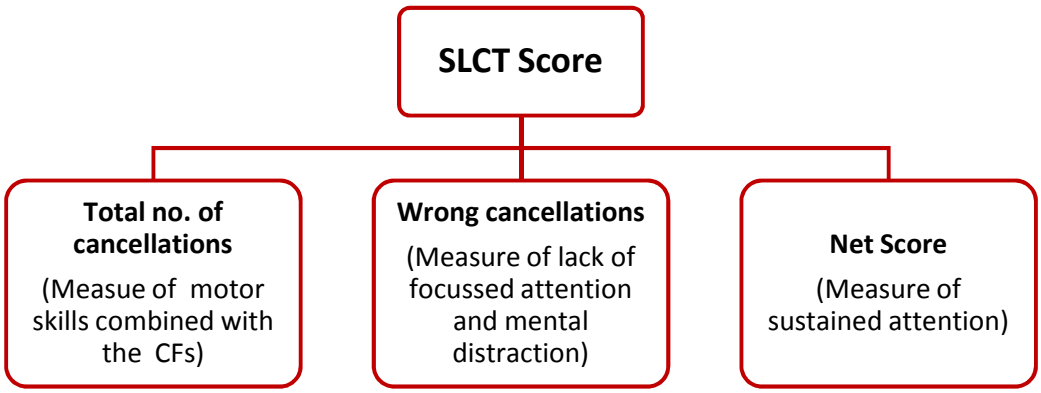

Figure 4.8: SLCT scoring

4.5.7.2 Stroop Test

The Stroop test is a cognitive function test to determine the executive functions of the individuals (Purohit and Pradhan, 2017). The test also determines the selective attention, cognitive flexibility and resistance to interference from other sources (Homack and Riccio, 2004). Figure 4.9 shows the components of the Stroop test.

The first page of the test contains three words "RED," "GREEN," and "BLUE," in five columns printed in black ink and repeated randomly. The second page consists of the "XXXX" printed in 5 columns, randomly printed in red, green, or blue color, totaling 100 times. Finally, the third page, the words "RED, "GREEN," and "BLUE" randomly in 5 columns and 20 rows; totaling 100 times; are printed randomly in red, green, or blue color such that in no case do the words and the color in which they are printed are the same. The sample lines of the three test pages are shown in Appendix VI.2.

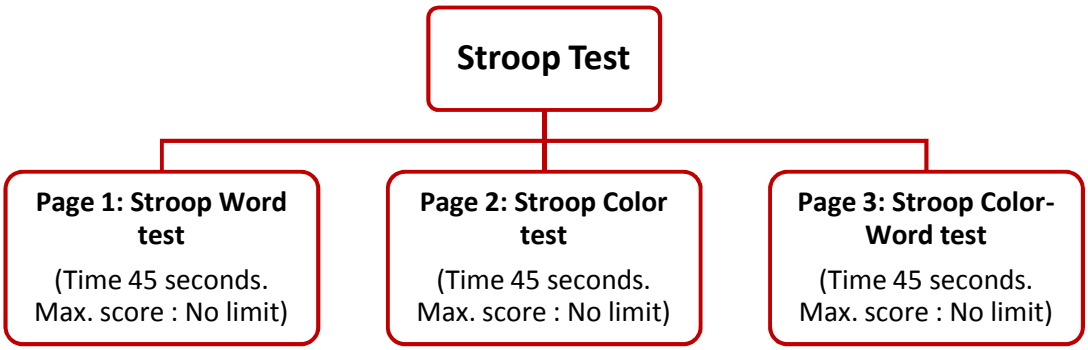

Figure 4.9: Components of the Stroop test

Stroop word test: During this test,the participant has to read the words printed, column-wise. The number of words read correctly in 45 seconds is the Stroop-word score (SWS).

Stroop color test: During this test,the participant has to say the color with which “XXXX” is printed on page 3 of the test, column-wise. If the whole page is read the participant continues to do the same again from the first column. The number of correct answers is the Stroop color score (SCS) for the participant.

Stroop color-word test: During this test, he participant has to say the color with which the word is printed (and not read the word) on page 3 of the test; which is also called the interference page, column-wise. If the whole page is done with, the same is done from the first column again. The number of correct colors of the word identified is the Stroop color-word score (SCWS) for the participant.

4.5.7.3 Digits Forward and Backward Span test (DSFB)

Digits Forward and backward digit span (DSFB) test is used for testing working memory (Jagannathan, Raghuram and Talwadkar, 2014). Figure 4.10 shows the overview of components of the DSFB test. The sample test sheet is shown inAppendix VI.2. The test sheet contains two sections, the first section for 'forward test' (DSF) having eight sets of numbers andthe second section for 'backward test' (DSB) having seven sets of numbers; with each set having two sequences of numbers referred to as trial-1 and trial-2. During the DSF, the examiner first reads out the sequence of numbers printed in the test sheet, starting with the first row. The participant is required to repeat the sequence of numbers in the same order. Then the process is repeated for trial-2. If the participant answers at-least one (or both) the trials correctly the test continues. One point is scored for each correct trial. The test is repeated for all the items. The maximum points scored is 16 points. After the DSF test, on the same lines, the participant undergoes the DSB test. The procedure and scoring for the DSB test except that when the examiner reads out the sequence of numbers the participant is required to repeat the numbers in reverse order. The maximum score including both DSF and DSB, i.e., the digit span total (DSTot) score is 16+14=30 points.

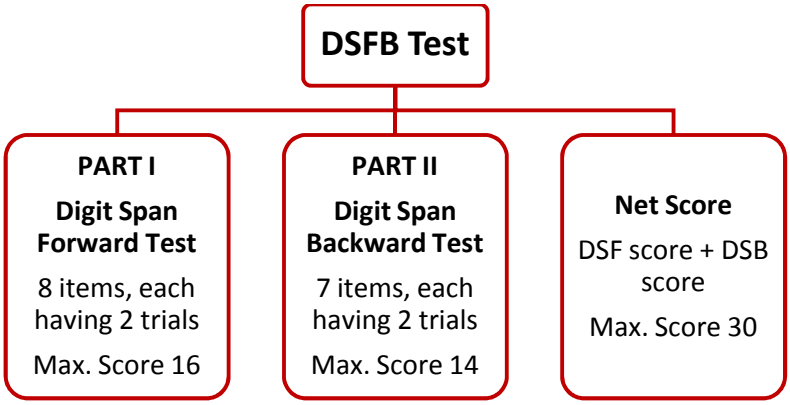

Figure 4.10: Digit Span Backward Forward test

4.5.7.4 Symbol Digit Modalities Test:

Symbol Digit Modalities Test (SDMT) is yet another test to determine the cognitive functioning of the participants. This test is a neuropsychological test for cognitive processing speed. The test also is influenced by visual acuity and ocular motor functions (Costa *et al.*, 2017). Appendix VI.2 shows the sample first few lines of the test sheet. The test sheet has nine symbols for nine digits, 1 to 9. The test sheet has the symbols randomly printed, with space for the participant for writing the corresponding number. The participant is required to see the symbol and write the number corresponding to each symbol. The test time is 90 seconds. The number of correct substitutions made is the score for the participant.