

Cerebrospinal Fluid Dynamics and Accumulation of Amyloid in the Brain Tissue of Rat, *Rattus Norvegicus* (L).

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Abstract-- Attempt is dealing with analysis of volume; production and rate of turnover of CSF with reference to accumulation of amyloid-beta-peptide in the brain of aging rat. CSF-production was assayed through Ventriculo-Cisternal-Perfusion along with “Blue-Dextran” in artificial CSF; CSF-volume through MRI; and CSF-rate of turnover (by dividing CSF-production rate by the volume of the space of CSF. The measurements of concentration of amyloid-beta peptides (Ab.40 and Ab.42) in the cortical tissue and hippocampus were carried through the method of ELISA. The significant and linear increase in the volume of CSF of cranium with age: 7-21 months ($p < 0.01$); 7-28 months ($p < 0.001$). The rate of production of CSF was reported to increase from 7-28 months ($p < 0.01$) and to decrease from 14-28 months ($p < 0.05$). There was initial increase in the turnover of CSF from 7 months to 21 months and then a decrease to 28 months of age. The concentration of amyloid-beta peptides (Ab.40 and Ab.42) in the brain increased from 7-28 months ($p < 0.001$). The steady state level in the concentration of amyloid-beta peptides (Ab.40 and Ab.42) was found approached by 28 months. No correlation was found between the concentration of amyloid-beta peptides (Ab.40 and Ab.42) and CSF-turnover in early aged (young) (7 – 14 months) rats.

Keywords-- Aging Related Amyloid-Beta-Peptides, CSF-turnover, Accumulation of Amyloid.

I. INTRODUCTION

Cerebro-Spinal-Fluid (C.S.F.) is a transparent (clear and colourless) trans-cellular-body-fluid present in the meninges around the brain and spinal-cord. It is also present within the brain-ventricles. The circulation of this fluid appears to be critical with reference to maintenance of the healthiest environment of the brain. The process of regular aging reflects on the functional decline in the quantity and qualities of this fluid and may leads to the conditions of “age-related dementias”. There is decrease in the rate of production (as well as turnover rate) of Cerebro-Spinal-Fluid (C.S.F.) in older age and diseased conditions.

The older age and diseased conditions are also related to the diminished rate of protein-clearance, peptide and other metabolites toxic categories, altered transport of ions and solutes, and decrease in the power of resistance to the stress of oxidative category [1-7]. Cerebro-Spinal-Fluid (C.S.F.) is blood-ultrafiltrate, as a secretion from the choroid-plexus (plica-choroidea) and under circulation for ventricles of the brain, the leptomeninges (the innermost layers of meninges and serving as membranes of protection around the brain and spinal cord). In human being, the circulation of Cerebro-Spinal-Fluid (C.S.F.) follows re-entering the bloodstream and the arachnoid villi [8]. The nasal lymphatics in rodents are concerned with absorption of the most significant amount of Cerebro-Spinal-Fluid (C.S.F.) [9]. The rate of complete replacement of volume of Cerebro-Spinal-Fluid (C.S.F.) is termed as CSF-turnover. This CSF-turnover deserves significance and playing role in in the process of clearance of solutes from the tissues pertaining brain and spinal cord [8]. Rubenstein E (1998) and Johanson, *et al* (2004) reported fifty percent decrease in the production of Cerebro-Spinal-Fluid (C.S.F.) through the choroid plexus with the age in human being and animal models [8,10]. The flattening condition of epithelial cells of choroid plexus; fibrotic scarring (or fibrosis) and increase in the inclusions of the cells are supposed to be concerned with decrease in the production of Cerebro-Spinal-Fluid (C.S.F.) through the choroid plexus with the age in human being and animal models [7, 10, 11]. Matsumae, *et al* (1996) reported increase in the production of Cerebro-Spinal-Fluid (C.S.F.) through the choroid plexus with the increasing age, accompanied by atrophy (progression in the loss of nerve cells leading shrinkage of tissues pertaining central nervous system) of the brain [12]. Therefore, the rate of production of Cerebro-Spinal-Fluid (C.S.F.) is divided by total volume of Cerebro-Spinal-Fluid (C.S.F.) to know the turnover of Cerebro-Spinal-Fluid (C.S.F.). The concentration of proteins in the Cerebro-Spinal-Fluid (C.S.F.) use to exert influence on the age-related turnover.

The turnover of Cerebro-Spinal-Fluid (C.S.F.) substantially decreases with age [13]. There is possibility of consequences pertaining clearance proteins and peptides of toxic category (and other metabolites too) through reduction in the turnover of Cerebro-Spinal-Fluid (C.S.F.). Chen, *et al* (2010) reported close relation between elevated levels in the albumin and decrease in the production (and turnover too) of Cerebro-Spinal-Fluid (C.S.F.) in older sheep [14]. The albumin is a soluble protein content of the Cerebro-Spinal-Fluid (C.S.F.). There are thirty-six to forty-three amino acids in the beta-amyloid (or amyloid-beta-peptide / Ab), the most significant components of the plaques of amyloid appearing in the brain tissue of patients of Alzheimer's disorder [15]. In a cognitively intact (mentally sound / alert) and standard type of normal aging person, there is inverse relation between the concentration of amyloid beta peptide (Ab) and accumulation of amyloid in brain [16, 17, 18]. According to Andreasen, *et al* (2001), the biomarker status for Alzheimer's disorder is "Lower than normal concentration of the amyloid-beta-peptide-forty-two" in Cerebro-Spinal-Fluid (C.S.F.) [19]. The similar type of relationship (inverse relationship) among the concentration of amyloid beta peptide (Ab) in parenchymal tissues and the Cerebro-Spinal-Fluid (C.S.F.) has been reported in the animal models of aging and the patients of Alzheimer's disorder (AD) [20, 21, 22]. There is no formation of fibril and plaque in rats of wild-type. Formation of fibrils and plaques appear only in human being. In case of wild rats, there is self-assembly of amyloid beta peptide (Ab) into oligomeric forms (consisting of a few repeating units) and self-aggregation in the parenchyma of brain as medium and proceed for the formation of large granules when concentration of amyloid beta peptide (Ab) uses to increase [23, 24].

The human subjects of "Non-Alzheimer-disorder" (Non-AD) also exhibits the conditions of "enlargement of cerebral-ventricles and decrease in turnover of the Cerebro-Spinal-Fluid (C.S.F.)" [25]. There are many reports in the pathological cases of Alzheimer's disorder exhibiting the symptoms with disease of "Normal-Pressure-Hydrocephalus". This disease of "Normal-Pressure-Hydrocephalus" is characterized by enlargement of ventricles and the condition of decrease in the turnover of the Cerebro-Spinal-Fluid (C.S.F.) [26]. Mawuenyega, *et al* (2010) used to determine clearance of amyloid beta peptide (Ab) and reported reduced condition of clearance of amyloid beta peptide (Ab) in patients of Alzheimer's disorder (AD) and unchanged condition in production of amyloid beta peptide (Ab) [27]. Serot, *et al* (2011) reported inverse relationship between clearance of amyloid beta peptide (Ab) and ventricular volume (and turnover of Cerebro-Spinal-Fluid / C.S.F. too) [28].

Aging appears to be only one and most significant risk factor for the genesis of the Alzheimer's disorder (AD) single most important risk factor for AD genesis [29]. There are no reports on the relationship among the dynamics of Cerebro-Spinal-Fluid (C.S.F.) and brain accumulation of amyloid beta peptide (Ab). On this much is background for planning the present attempt on the dynamics of CSF and accumulation of amyloid in the brain tissue of rat, *Rattus norvegicus* (L).

II. MATERIAL AND METHODS

The attempt on "Dynamics of CSF and Accumulation of Amyloid in the Brain Tissue of Rat, *Rattus norvegicus* (L)." has been completed through the steps: (A). Laboratory Rearing of brown rat, *Rattus norvegicus* (L); (B). Measurement of the volume of Cerebro-Spinal-Fluid (C.S.F.) (both, cranial and ventricular); (C). Calculations pertaining production of Cerebro-Spinal-Fluid (C.S.F.), its rate of turnover and (D). Bioassay of accumulation of amyloid-beta-peptide (Ab) in the parenchyma of the brain and (E). Analysis of collected data through statistical methods.

(A). *Laboratory-Rearing of Experimental Animals Brown-rat, Rattus norvegicus* (L): For the present attempt on "Dynamics of CSF and Accumulation of Amyloid in the Brain Tissue of Rat, *Rattus norvegicus* (L), twenty-five adult females (twelve-weeks-old) and twenty-five adult males (twelve-weeks-old) Brown rats (*Rattus norvegicus* L) were housed in cages (one male and one female in each cage) in the laboratory of Dr APIS Laboratory. The weight of the experimental animals housed in cages was ranging from 160 gm to 200 gm. They were procured from the ICAR-National Institute of Abiotic Stress Management (NIASM), Malegaon, Baramati 413 115, Pune Maharashtra, India. The experimental animals (rats) were housed in quiet cages (20 - 25°C; 50 - 60% relative humidity). They were kept in laboratory with a condition of "12 hours light/dark cycle (7 a.m. - 7 p.m.)". They were fed with a commercial standard rat diet (Abaliogu YemSanayi, Denizli, Turkey) and water ad libitum. All animal procedures were approved by the Animal Care and Use Protocol (Department of Zoology, Shardabai Pawar Mahila Mahavidyalaya, Sharnagar Baramati). The experimental animals (brown rat, *Rattus norvegicus*) were reared in the laboratory through the method described by Vitthalrao B. Khayde and Apurva Baban Tamhane (2021) [30]. Continuous five generations of the individuals of experimental animals were maintained in laboratory conditions.

Male and female rats aged three months; twelve months; twenty months and thirty months from each generation were utilized for bioassay of the Cranial and Ventricular Cerebro-Spinal-Fluid (C.S.F.) and for the bioassay of amyloid-beta-peptide (Ab). The twenty male and twenty female (n = 40) experimental animals (brown-rat, *Rattus norvegicus* L.) utilized for measurement production of Cerebro-Spinal-Fluid (C.S.F.) (n = 40) were in a separate group from those utilized for measurement of volume of Cerebro-Spinal-Fluid (C.S.F.) (n = 40) and Bioassay of amyloid-beta-peptide (Ab) (n = 40). The experimental animals (brown-rat, *Rattus norvegicus* L.) were processed to euthanize after the completion of acute experimentation. The method of intraperitoneal pentobarbital (one hundred-twenty-five milligram per kilogram) was followed for the purpose to euthanize the experimental animals (brown-rat, *Rattus norvegicus* L.). The experimental animals (brown-rat, *Rattus norvegicus* L.) utilized for ELISA (Enzyme-Linked Immunosorbent-Assay) measurement were processed for perfusion with phosphate-buffer-saline (PBS) through the left ventricular-cannulation before the removal of the brain. The purpose of selecting brown-rat, *Rattus norvegicus* (L) as experimental animals is their long life and their availability at most age (up-to thirty months) from the ICAR-National Institute of Abiotic Stress Management (NIASM), Malegaon, Baramati 413 115, Pune Maharashtra, India.

(B). *Measurement of the volume of Cerebro-Spinal-Fluid (C.S.F.) (both, cranial and ventricular)*: Male and female rats aged three months; twelve months; twenty months and thirty months from each generation were utilized for bioassay of the Cranial and Ventricular Cerebro-Spinal-Fluid (C.S.F.). The purpose of selecting brown-rat, *Rattus norvegicus* (L) as experimental animals is their long life and their availability at most age (up-to thirty months) from the ICAR-National Institute of Abiotic Stress Management (NIASM), Malegaon, Baramati 413 115, Pune Maharashtra, India. “Siemens-3T-TIM(Total-Imaging-Matrix)-Trio-MR-scanner” was utilized for the purpose of measurement of Cerebro-Spinal-Fluid (C.S.F.) (both, Cranial and Ventricular). “Siemens-3T-TIM(Total-Imaging-Matrix)-Trio-MR-scanner” is a high-performance (3.0 Tesla) whole body magnetic resonance imaging (MRI) scanner. It is mostly regarded for clinical application as well as for research. Distinguishing feature of this “Siemens-3T-TIM(Total-Imaging-Matrix)-Trio-MR-scanner” is presence of strong SNR (Signal-to-Noise-Ratio) and flexibility in the system of coil-technology. The “Siemens-3T-TIM-Trio-MR-scanner” was used to equip with an AC-88 high-performance-gradient-insert and custom-volume-transmit-receive-coil 4.5 cm).

The experimental animals brown-rat, *Rattus norvegicus* (L) were processed for imaging through the condition of continuous anaesthesia. The volatile and halogenated ether, “Isoflurane” was utilized for anaesthesia. “Isoflurane” is mostly used as a common purpose of inhalation anaesthetic formulation during surgical events. “Isoflurane” is known to induce (and to maintain too) general unconsciousness during surgical events. The T1 weighted Magnetization Prepared Rapid Gradient Echo (MPRAGE) were used to acquire (thickness of slice: 0.2 milli-meter; view-field: 6.0 centimeter; Matrix: 320 cubic milli-meter, Repetition Times / TR: 2700 ms, Time Echo / TE: 3.89 ms, Time of inversion: 900 ms, angle of flip: nine degrees, time of acquisition: twelve minutes thirty-eight second) for the total eight acquisitions. The acquisitions were processed for alignment to the images in original form. The acquisitions were averaged through the use of the AFNI (Analysis of Functional Neuroimages) package of the software [31]. Images were used to import as NIFTI (Neuroimaging Informatics Technology Initiative) files into the image-analyser, Slicer-3D [32, 33, 34]. Slicer-3D (source of software) include the brightness / contrast function; normal zoom function and a pixel-by-pixel magnifying glass. This magnifying glass uses to allow the user for the purpose of the determination that pixels in the images of T1-weighted are grey or black. Fiducial marker points were used to place only on black type of the pixels. From the images of fiducial-marked category, the “Fast-Marching-Segmentation” module uses to build a model of three dimension (3D) from surrounding pixels with a similar grey-scale-value [35]. In each slice, the spaces for ventricular cerebro-spinal-fluid (CSF) and spaces of subarachnoid category were marked with fiducial - points. (The list of the spaces of ventricular cerebro-spinal-fluid / CSF: right lateral ventricle; left lateral ventricle; the third ventricle / a narrow mid-line cavity situated in between left half and right half of thalamus, aqueduct of Sylvius or cerebral aqueduct and the fourth ventricle / diamond shaped cavity between brain-stem and cerebellum). The total volume cerebro-spinal-fluid (CSF) in cranium was calculated from the sum of cerebro-spinal-fluid (CSF) in the ventricles and cerebro-spinal-fluid (CSF) in the subarachnoid spaces. For this purpose, the “Fast-Marching-Segmentation-Module”. The Fast-Marching-Segmentation-Module is a machine or a tool of semi-automatic category used for processing the images through the propagation of waves to the segment-anatomical-regions. Through the process to place the starting-point (or seed), the algorithm uses to propagate advancing front (such as a boundary / contour outward based on the intensity of image and local gradient volumes of cerebro-spinal-fluid (CSF) through the use of the Fast-Marching-Segmentation-Module).

The analysis of volume was carried out through the use of “one reader” for the purpose of consistency in the results.

(C). *Calculations pertaining production and rate of turnover of Cerebro-Spinal-Fluid (C.S.F.)*: The method described by Chodobski A and Segal M. (2005) was followed for the purpose of calculations pertaining production and rate of turnover of Cerebro-Spinal-Fluid (C.S.F.) [36]. The experimental animals brown-rat, *Rattus norvegicus* (L) were processed for anaesthetization with the help of intraperitoneal (pentobarbital). The anaesthetization allowed the experimental animals brown-rat, *Rattus norvegicus* (L) for immobilization in frame of stereotaxic position. Thereafter, pair of cannulae (stainless-steel; gauge: 27) was used to insert (one into each of the lateral-cerebral-ventricle). “Blue-Dextran-2000” (chemical reagent with Cibacron blue / F.3.G.A.) @ five milligram per milli-litre was used for perfusion as a mock Cerebro-Spinal-Fluid (C.S.F.) through each cannulae @ two micro-litre per minute. Insertion of third-cannula was followed into the “Cisterna-Magna”. Insertion of third-cannula into the “Cisterna-Magna” was for the purpose to collect the Cerebro-Spinal-Fluid (C.S.F.). The pressure pertaining “Intraventricular” was used to monitor through the three-way fittings (T-connectors) to the cannulae of infusion. The samples (Cerebro-Spinal-Fluid / C.S.F.) were used to collect at the interval of twenty minutes. The determination of the concentration of “Blue-Dextran-2000” was processed through the use of spectrophotometer, through the measurement of optical density (absorbance) at 620 nano-metre (nm). The mathematical formula used for the purpose of calculation of rate of formation of Cerebro-Spinal-Fluid (C.S.F.) was as below: $V.F. = V.I \times [(C.I. - C.O) \div C.O]$. Here, in this mathematical formula, V” is representing the rate of flow of Cerebro-Spinal-Fluid (C.S.F.); “C” is representing the concentration of dye, “Blue-Dextran-2000”; (F: Formation; I: inflow; O: out-flow) [36]. For the purpose of calculation of turnover of Cerebro-Spinal-Fluid (C.S.F.), mean production of Cerebro-Spinal-Fluid (C.S.F.) was divided by the mean volume (microlitre) of Cerebro-Spinal-Fluid (C.S.F.) in each age group in attempt. The figure thus obtained was multiplied by 1440 minutes per day. The final figure was labelled as value of turnover of the Cerebro-Spinal-Fluid (C.S.F.) per day.

(D). *Bioassay of accumulation of amyloid-beta-peptide (Ab) in the Parenchyma of the Brain*: The phosphate-buffered-saline (PBS) was utilized for the purpose of excision of F.P cortex (Fronto-Parietal-Cortex) and excision of “Formation of Complete Hippocampus” from the post-perfused brains of the experimental animal brown-rat, *Rattus norvegicus* (L).

The collected samples were then processed for grinding thoroughly into the form of fine powder. The liquid-nitrogen-bed was utilized for grinding the collected samples. The weight of sample (in powder form) used for further the homogenization and extraction of the proteins. Concentrations of amyloid-beta peptides (Ab.40 and Ab.42) in cortex and hippocampus of the samples of brains the experimental animal brown-rat, *Rattus norvegicus* (L) was measured through the use of method of “Sandwich Enzyme Linked Immunosorbent Assay” (Sandwich-ELISA) (as described by Silverberg, *et al*, 2010) [37]. High-sensitivity kit of ELISA (Rat VEGF ELISA Kit catalogue No. EK0540 / 1610 Faraday Ave Carlsbad 92008) was selected for the purpose to reduce background and for the purpose of improvement in detecting amyloid-beta peptide (Ab.40) (sensitivity: 0.049 pico-mol per litre) and amyloid-beta peptide (Ab.42) (sensitivity: 0.024 pico-mol per litre). BNT77 is a mouse-monoclonal-antibody. It uses to target specifically, the central region of amino acids of amyloid beta peptide (Ab). This BNT77 monoclonal antibody directed against, “Ab11- 28” was used as the “Capture Antibody”. It is designed to detect both full-length amyloid beta peptide (Ab) as well as amyloid beta peptide (Ab) with a truncated (made shorter through cutting) or modified N-terminus. For specific detection of amyloid beta peptide (Ab40), the BA27 (monoclonal antibody) directed against “C-terminal-portion” of Ab40 was utilized. And for specific detection of amyloid beta peptide (Ab42), the BC05 (monoclonal antibody) directed against “C-terminal-portion” of Ab42 was utilized. The contents of total-protein in each collected sample were determined through the classical method of Lowry, *et al* (1951) [38]. The contents of amyloid-beta peptide (Ab) were expressed in the unit of pico-gram per mg total proteins.

(E). *Analysis of collected data through statistical methods*: The whole experimentations were repeated for three times for the purpose to obtain consistency in the results. All the events pertaining data-analysis, the “Graph-Pad-Prism-v4.01 (Graph-Pad-Software, San-Diego, CA, USA) was utilized. Data figures for the volume of Cerebro-Spinal-Fluid (CSF) and its production were the first subject for analysis of variance (ANOVA) and then to Bonferroni's-multiple-comparison-test. The turnover of Cerebro-Spinal-Fluid (CSF) was followed for calculation for each age group of the experimental animal brown-rat, *Rattus norvegicus* (L) from the means of the figures of the production of Cerebro-Spinal-Fluid (CSF) and the CSF volume of Cerebro-Spinal-Fluid (CSF) measurements. The statistical significance was used for setting at $p < 0.05$.

III. RESULTS AND DISCUSSION

Results on Dynamics of CSF and Accumulation of Amyloid in the Brain Tissue of Rat, *Rattus norvegicus* (L) are presented in tabular form (table No.1,2,3, 4, 5 and 6) and presented through plotting the graphs (figures: 1,2, 3, 4, 5 and 6). The ventricular (lateral ventricles, third ventricle, and the fourth) volume of Cerebro-Spinal-Fluid (C.S.F.) (Microlitre) in male Brown-rat, *Rattus norvegicus* (L) of 7, 14, 21 and 28 months old was 7.368 (± 01.786); 15.789 (± 04.739); 29.584 (± 05.263) and 33.684 (± 06.315) units respectively (table 1). The ventricular volume of Cerebro-Spinal-Fluid (C.S.F.) (Microlitre) in female Brown-rat, *Rattus norvegicus* (L) of 7, 14, 21 and 28 months old was 6.557 (± 0.631); 14.036 (± 0.789); 26.329 (± 1.071) and 29.978 (± 3.694) units respectively (table 1). Volume of Ventricular Cerebro-Spinal-Fluid (C.S.F.) (Microlitre) in male and female Brown-rat, *Rattus norvegicus* (L) in present attempt exhibiting the tendency of significant linear increase with the age of experimental animals.

The cranial volume of Cerebro-Spinal-Fluid (C.S.F.) (Microlitre) in male Brown-rat, *Rattus norvegicus* (L) of 7, 14, 21 and 28 months old was 132.57 (± 52.631); 197.36 (± 65.789); 289.47 (± 59.071) and 394.73 (± 78.694) respectively (table 2). The cranial volume of Cerebro-Spinal-Fluid (C.S.F.) (Microlitre) in female Brown-rat, *Rattus norvegicus* (L) of 7, 14, 21 and 28 months old was 116.66 (± 23.476); 174.036 (± 29.773); 256.73 (± 27.124) and 349.33 (± 38.786) units respectively (table 2). The cranial volume of Cerebro-Spinal-Fluid (C.S.F.) (Microlitre) in male and female Brown-rat, *Rattus norvegicus* (L) in present attempt exhibiting the tendency of significant linear increase with the age of experimental animals in the attempt.

Production of Cerebro-Spinal-Fluid (C.S.F.) (Microlitres per minute) in male Brown-rat, *Rattus norvegicus* (L) of 7, 14, 21 and 28 months old was 00.465 (± 00.063); 03.128 (± 00.419); 02.321 (± 00.374) and 00.928 (± 00.077) units respectively (table 3). Production of Cerebro-Spinal-Fluid (C.S.F.) (Microlitres per minute) in female Brown-rat, *Rattus norvegicus* (L) of 7, 14, 21 and 28 months old was 00.417 (± 00.059); 02.723 (± 00.486); 02.319 (± 00.453) and 00.819 (± 00.072) units respectively (table 3). Production of Cerebro-Spinal-Fluid (C.S.F.) (Microlitres per minute) in male Brown-rat, *Rattus norvegicus* (L) of 7, 14, 21 and 28 months old was found varying significantly with the age of experimental animals in the attempt.

Turnover of Cerebro-Spinal-Fluid (C.S.F.) (Litre per day) in male Brown-rat, *Rattus norvegicus* (L) of 7, 14, 21 and 28 months old in the attempt was recorded 07.346 (± 00.672); 11.739 (± 00.836); 09.164 (± 00.518) and 01.479 (± 00.268) respectively (table 4).

Turnover of Cerebro-Spinal-Fluid (C.S.F.) (Litre per day) in female Brown-rat, *Rattus norvegicus* (L) of 7, 14, 21 and 28 months old in the attempt was recorded 06.598 (± 00.637); 10.218 (± 00.715); 08.156 (± 00.619) and 00.786 (± 00.013) respectively (table 4). Turnover of Cerebro-Spinal-Fluid (C.S.F.) (Litre per day) in Brown-rat, *Rattus norvegicus* (L) of 7, 14, 21 and 28 months old in the attempt is calculated through the use of mean values of pertaining attempts of production and the volume measurement. Both (production of Cerebro-Spinal-Fluid / C.S.F. / Microlitres per minute and the turnover of Cerebro-Spinal-Fluid / C.S.F. / Litre per day) in Brown-rat, *Rattus norvegicus* (L) in the attempt exhibited non-linear tendency and show initial increase to fourteen months of age with subsequent decrease to thirty months of age. There was a significant value of decrease in the turnover of Cerebro-Spinal-Fluid (C.S.F.) (Litre per day) in Brown-rat, *Rattus norvegicus* (L) between twenty-first and twenty-eighth months in comparison with the rate of turnover rate of Cerebro-Spinal-Fluid (C.S.F.) (Litre per day) prior to twenty-one months.

The healthy brown-rats, *Rattus norvegicus* (L) use naturally to produce (as well as to store) the amyloid-beta-proteins (or peptides / Ab) of intracellular category in the regions of brain (entorhinal-cortex and hippocampus). There is quantitative similarity in baseline expression between brain of healthy rats and brain of healthy human being (though it uses rarely to form extracellular-plaques of harmful category). The concentration of beta amyloid protein (peptide) (Ab40) in the Cerebro-Spinal-Fluid (C.S.F.) (Unit: picogram per milligram) in male Brown-rat, *Rattus norvegicus* (L) of 7, 14, 21 and 28 months old in present attempt was found measured 04.328 (± 00.547); 05.876 (± 00.589); 07.097 (± 00.693) and 07.786 (± 00.835) units respectively (table 5). The concentration of beta amyloid protein (peptide) (Ab40) in the Cerebro-Spinal-Fluid (C.S.F.) (Unit: picogram per milligram) in female Brown-rat, *Rattus norvegicus* (L) of 7, 14, 21 and 28 months old in present attempt was found measured 3.875 (± 00.971); 05.261 (± 00.758); 06.328 (± 00.682) and 07.297 (± 00.739) units respectively (table 5). The most significant difference was recorded in between seven and fourteen months; seven and twenty-one months. It was followed by decrease in rate of rising tendency in beta amyloid protein (peptide) (Ab40) between fourteen and twenty-eight months. There was tendency of approaching a steady state in between twenty and twenty-eight months.

The concentration of beta amyloid protein (peptide) (Ab42) in the Cerebro-Spinal-Fluid (C.S.F.) (Unit: picogram per milligram) in male Brown-rat, *Rattus norvegicus* (L) of 7, 14, 21 and 28 months old in present attempt was found measured 01.991 (± 00.879); 05.614 (± 00.786); 08.318

(± 00.921) and 09.823 (± 01.427) units respectively (table 6). The concentration of beta amyloid protein (peptide) (Ab42) in the Cerebro-Spinal-Fluid (C.S.F.) (Unit: picogram per milligram) in female Brown-rat, *Rattus norvegicus* (L) of 7, 14, 21 and 28 months old in present attempt was found measured 1.782 (± 00.786); 05.026 (± 00.714); 07.743 (± 00.891) and 08.951 (± 00.894) units respectively (table 6). The beta amyloid protein (peptide) (Ab42) was found continued tendency of rising to twenty-first month. Afterward, the rate of increase was found slowed down to twenty-eighth month. The ratio of beta amyloid protein (peptide) (Ab40) to beta amyloid protein (peptide) (Ab42) in the brain-parenchyma was found reversal tendency with the advancement in the age.

The volumes of brain of the adult male brown-rat, *Rattus norvegicus* (L) is larger than the adult female brown-rat, *Rattus norvegicus* (L). The weight of adult male brown-rat, *Rattus norvegicus* (L) is 2.00 grams and that of the adult female brown-rat is about 1.786 grams, *Rattus norvegicus* (L). However, with reference to overall size of the body, relative sizes of brain and proportions of the subregions, it exhibits significant specific-variations. There are no reports on the fixed differences in the total volume of CSF (cerebrospinal fluid) between male brown-rat, *Rattus norvegicus* (L) and female brown-rat, *Rattus norvegicus* (L). The total volume of CSF (cerebrospinal fluid) uses to correlate directly with the weight, age, and size of the brain and not with the sex of individual rat. There is significant variation between skull size of male rat and female rat. Therefore, absolute volume of CSF (cerebrospinal fluid) often differs. (This difference is not due to sex specific scaling with reference to physiology). Total volume of Cerebro-spinal-Fluid (CSF) in brown rat, *Rattus norvegicus* (L), is ranging from 200 - 275 microlitres (μ l). This status of total volume of Cerebro-spinal-Fluid (CSF) in brown rat, *Rattus norvegicus* (L) is depending on age and weight. For researchers, it is possible to withdraw 50 to 180 microlitres (μ l), from the cisterna magna in a single-attempt of collection of samples of Cerebro-spinal-Fluid (CSF) in brown rat, *Rattus norvegicus* (L). Murtha, et al (2014) [39], reported 3.7 microlitres (μ l) of Cerebro-spinal-Fluid (CSF) per minute in brown rat, *Rattus norvegicus* (L) continually through the choroid-plexus. The volume of the Cerebro-spinal-Fluid (CSF) uses to remain proportional to the size of body size. The present attempt has tried its best to identify sex-based specificity in the differences in the composition and dynamics of Cerebro-spinal-Fluid (CSF). Analysis pertaining the proteomic shows variations in protein-composition.

The expression of genes pertaining some choroid-plexus for example, use to exhibit difference between individuals, which results in regulation of certain proteins in the pattern of up and (or) down in male individuals of rat in comparison with the female individuals of rat. During certain stressors pertaining physiology (example: diets of high-fat diet), there is possibility of exhibiting higher rate of secretion and different drainage-resistance of Cerebro-spinal-Fluid (CSF) by the female individuals of rats (in comparison with male individuals of rats). Baseline flow-velocity (measurements pertaining aqueduct) of Cerebro-spinal-Fluid (CSF) is appearing similar among healthy-young-adult male rats and female rats. However, reduction in the flow-velocity of the Cerebro-spinal-Fluid (CSF) pertaining age, significantly affect in the older individuals of male rats [39, 40, 41, 42, 43]. The present attempt relates the volume of Cerebro-spinal-Fluid (CSF), its production and turnover with the concentration of beta amyloid protein (peptide) (Ab40 and Ab42). The pattern of reversal in the ratio of the concentration of beta amyloid protein (peptides) (Ab40 and Ab42) appears to be the key findings of the present attempt.

Determination of the significant causes of accumulation of beta amyloid protein (peptide) (Ab40 and Ab42) in the Cerebro-spinal-Fluid (CSF) with advancement of the age seems to be crucial with reference to the establishment of the most effective therapy for treatment to the patients of Alzheimer-disorder (AD). Imbalance between production and clearance of concentrations of beta amyloid protein (peptide) (Ab40 and Ab42) get results into the condition of disorder of Alzheimer (AD) [27]. Earlier reports are suggesting that, overall accumulation of beta amyloid protein (peptide) (Ab40 and Ab42) in aging individuals is not the result through the elevation in the concentration of beta amyloid protein (peptide) (Ab40 and Ab42), but rather a reduction in the rate of process of clearance of beta amyloid protein (peptide) (Ab40 and Ab42) from the Cerebro-spinal-Fluid (CSF) [27, 37, 44]. The significant pathways for the process of clearance of beta amyloid protein (peptide) (Ab40 and Ab42) include: receptors (at barrier between blood and brain) mediated active-transport; receptors (at the barrier between blood and the Cerebro-Spinal-Fluid / C.S.F.); enzymatic degradation (*in situ*) and bulk-flow and turnover of the Cerebro-Spinal-Fluid (C.S.F.). There is significant reduction in the beta amyloid protein (peptide) “(Ab)-efflux-transporter-expression” with the age. There is also significant elevation in the “Influx-Transporter-Expression” at the “Blood-Brain-Barrier” (BBB) with the age [37, 44].

It is Interesting to note here that, the trend in opposite manner appears to be true with reference to the beta amyloid protein (peptide) “(Ab)-Transporter-Expression” at the “Blood-CSF-Barrier (BCSFB). It may be response of “Compensatory-Type” for the overall reduction in “Blood-Brain-Barrier” (BBB) clearance of beta amyloid protein (peptide) (Ab) [45]. Turnover of the Cerebro-spinal-Fluid (CSF), therefore, may continue to increase most significant for clearance of the metabolites in the brain of aging individual rat [46]. The present attempt is demonstrating non-significant (as good as nil) relationship between accumulation of beta amyloid protein (peptide) (Ab) in the brain-tissue and rates of turnover of Cerebro-spinal-Fluid (CSF) in the rats of young age. There is possibility of inverse type of relationship between accumulation of beta amyloid protein (peptide) (Ab) in the brain-tissue and rates of turnover of Cerebro-spinal-Fluid (CSF) in elderly individuals of rat, as part of “Age-Related-Multi-Factorial” reduction in the clearance of beta amyloid protein (peptide) (Ab) through “Blood-Brain-Barrier” (BBB) and the systems of Cerebro-spinal-Fluid (CSF). The results of present attempt are demonstrating, “Increase in the concentration of beta amyloid protein (peptide) (Ab)” with the age. Production of Cerebro-spinal-Fluid (CSF) and turnover of Cerebro-spinal-Fluid (CSF) are increased up to fourteen months of age of experimental animals, brown-rat, *Rattus norvegicus* (L). This result in the present attempt appears to be parallel with Preston (2001) [7]. There is possibility of contribution of defects in age-dependent clearance of beta amyloid peptides (Ab) and Cerebro-spinal-Fluid (CSF) turnover in association to the overall elevation in the concentration of beta amyloid peptides (Ab) with advancement in the age. This temporal type of association between beta amyloid peptides (Ab) and Cerebro-spinal-Fluid (CSF)-dynamics in the aged (older) individuals of rat is not sufficient to prove significant relationship of the “Cause and Effect” category. No doubt, there is significant scope to conclude (or to argue) that the decrease in turnover of Cerebro-spinal-Fluid (CSF) is the most significant contributing-factor.

IV. CONCLUSION

The present attempt is reporting crucial influence of age on the parameters of Cerebro-Spinal-Fluid (CSF). There is elevation in the production and turnover of Cerebro-spinal-Fluid (CSF) from seven months to fourteen months in the brown rat, *Rattus norvegicus* (L) (Model: Rat-Aging). No relationship observed among the deposition of amyloid beta peptides (Ab); production and turnover of Cerebro-spinal-Fluid (CSF) before fourteen months old experimental animals.

The turnover of Cerebro-spinal-Fluid (CSF) is found related inversely to the accumulation of amyloid beta peptides (Ab) in the experimental animals of elderly age. The reduction in the rate of turnover of Cerebro-spinal-Fluid (CSF) in the experimental animals of older age in the present attempt is suggesting possible target for therapy to improve the status of health with reference to Cerebro-spinal-Fluid (CSF).

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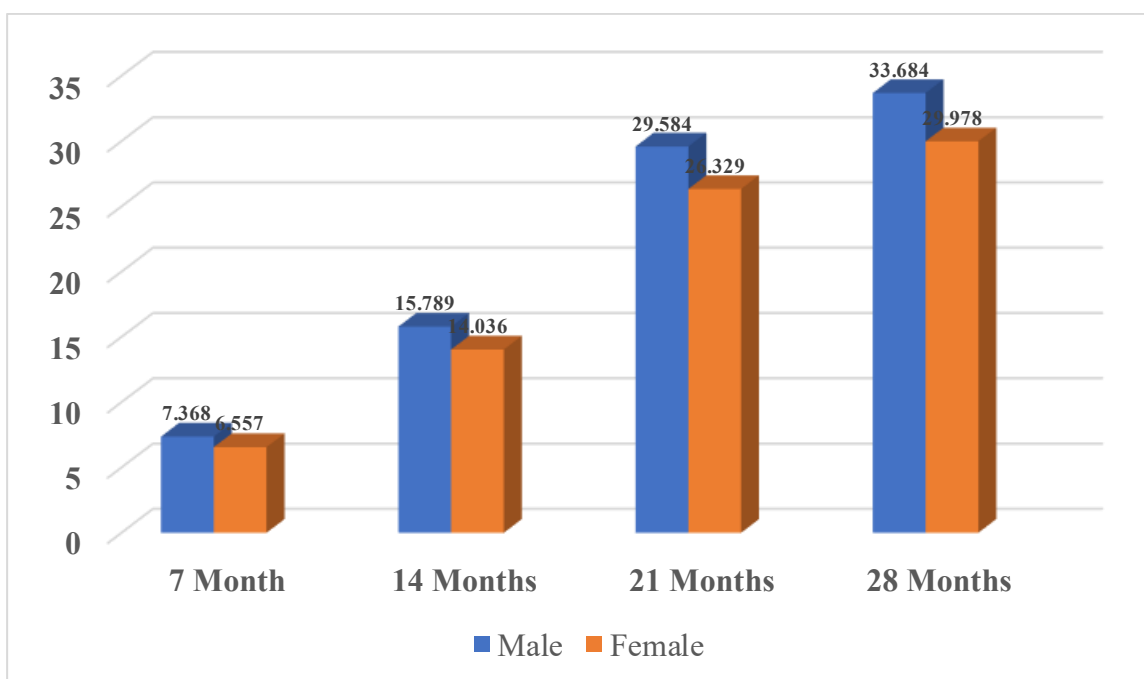


Fig. – 1: Age (months) and Ventricular Volume of Cerebro-Spinal-Fluid (C.S.F.) (Microlitre) in Male (n=25) and Female (n=25) Brown-rat, *Rattus norvegicus* (L).

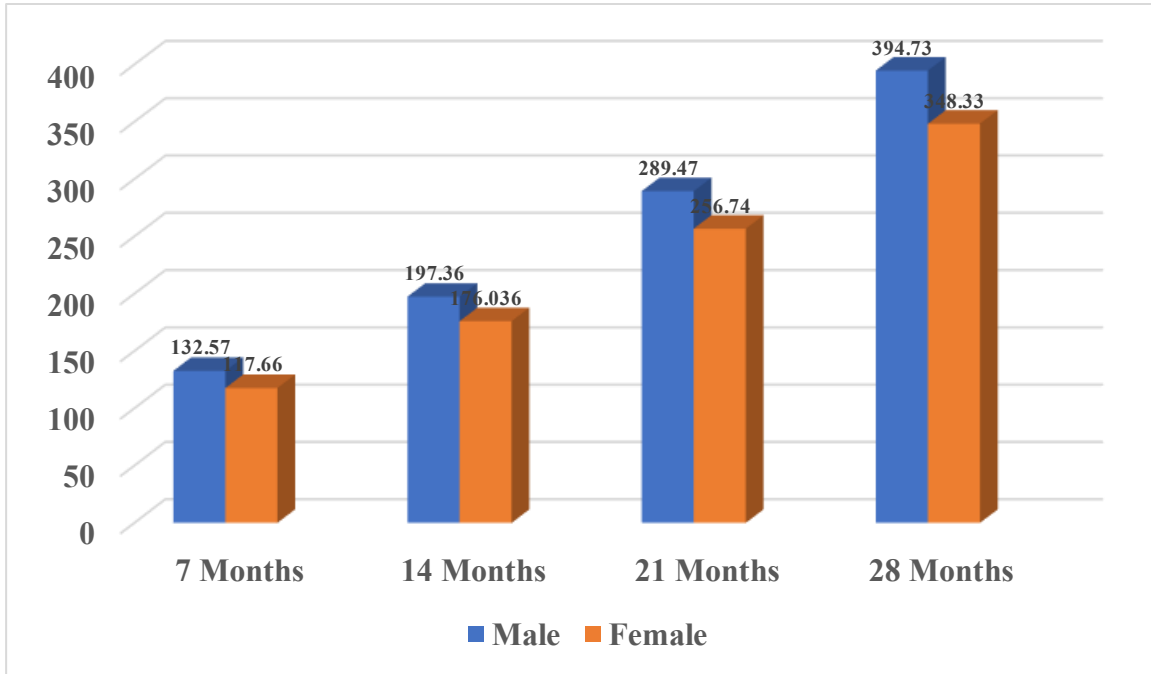


Fig. – 2: Age (months) and Total Cranial Volume of Cerebro-Spinal-Fluid (C.S.F.) (Microlitre) in Male (n=25) and Female (n=25) Brown-rat, *Rattus norvegicus* (L).

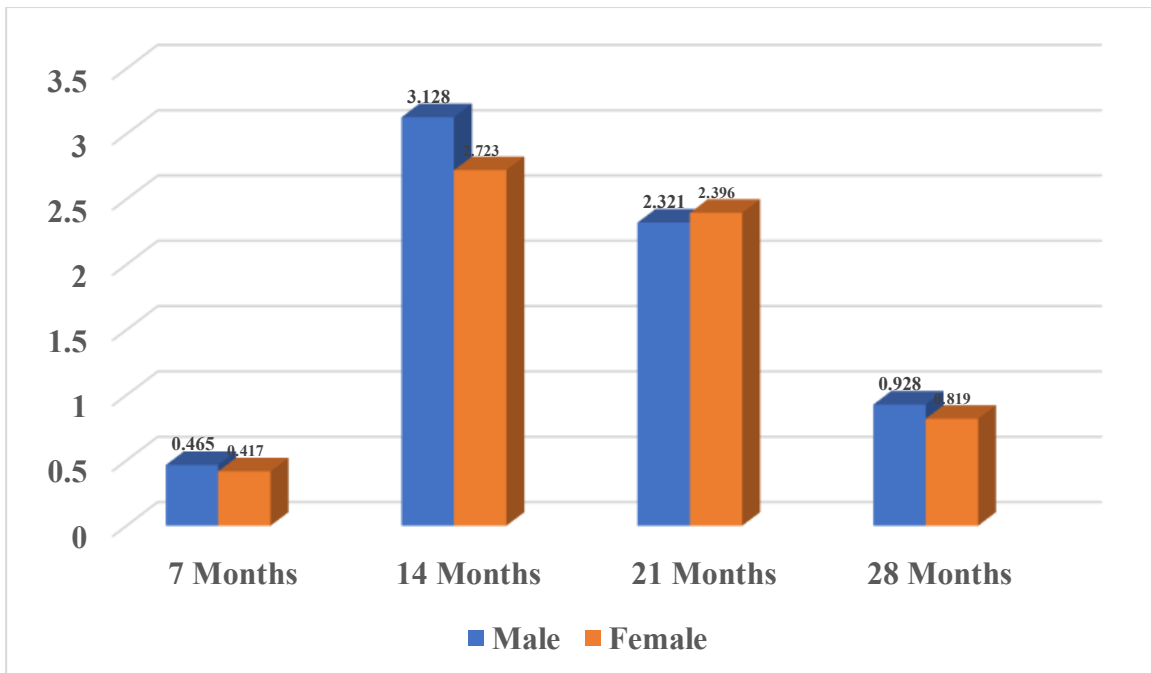


Fig. – 3: Age (months) and Production of Cerebro-Spinal-Fluid (C.S.F.) (Microlitre) in Male (n=25) and Female (n=25) Brown-rat, *Rattus norvegicus* (L).

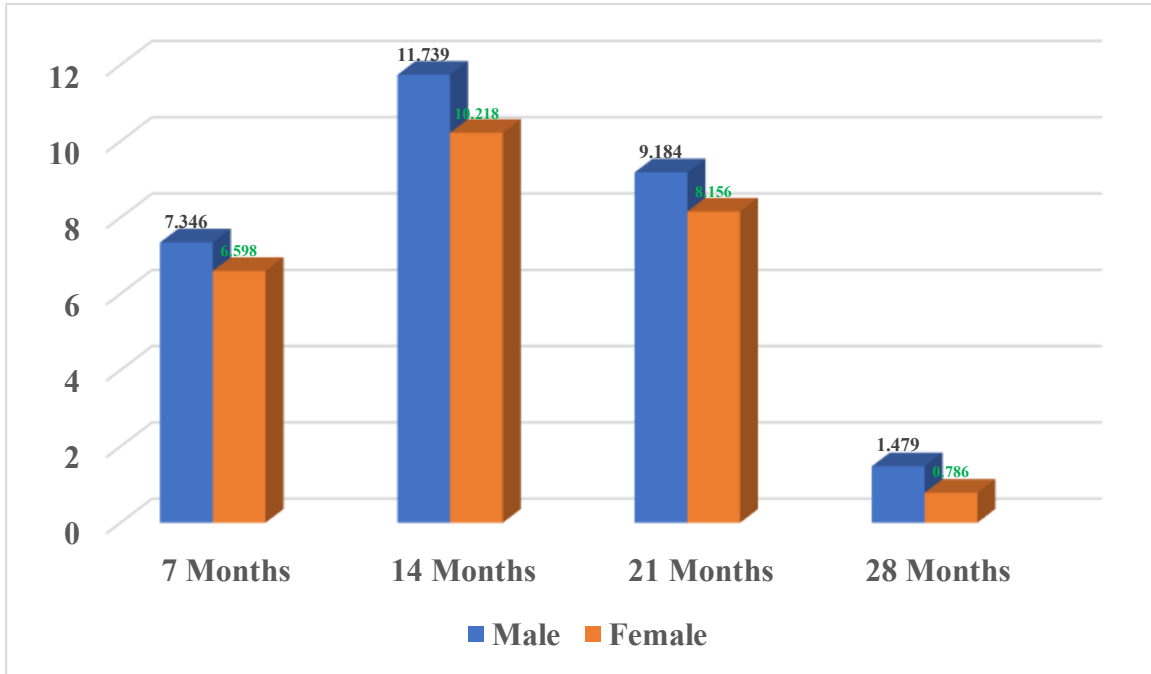


Fig. – 4: Age (months) and Turnover of Cerebro-Spinal-Fluid (C.S.F.) (Litre per day) in Male (n=25) and Female (n=25) Brown-rat, *Rattus norvegicus* (L).

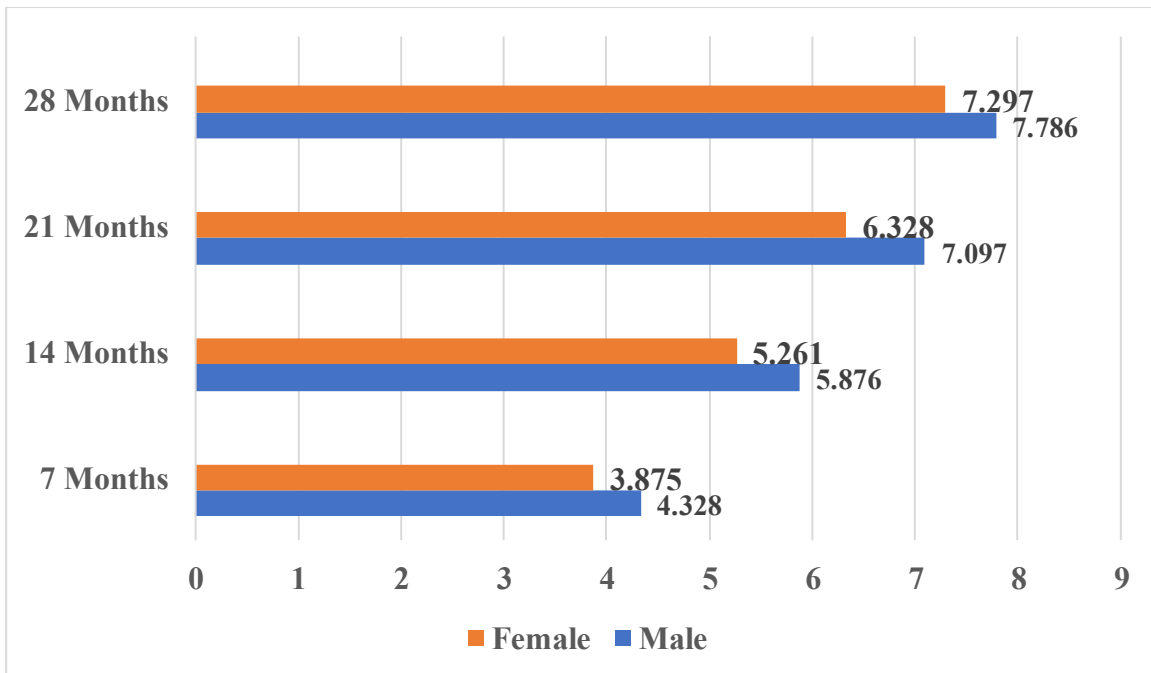


Fig. – 5: Age (months) and Concentration of Beta Amyloid Peptides (Ab40) in the Cerebro-Spinal-Fluid (C.S.F.) (Unit: picogram per milligram) in Male (n=25) and Female (n=25) Brown-rat, *Rattus norvegicus* (L).

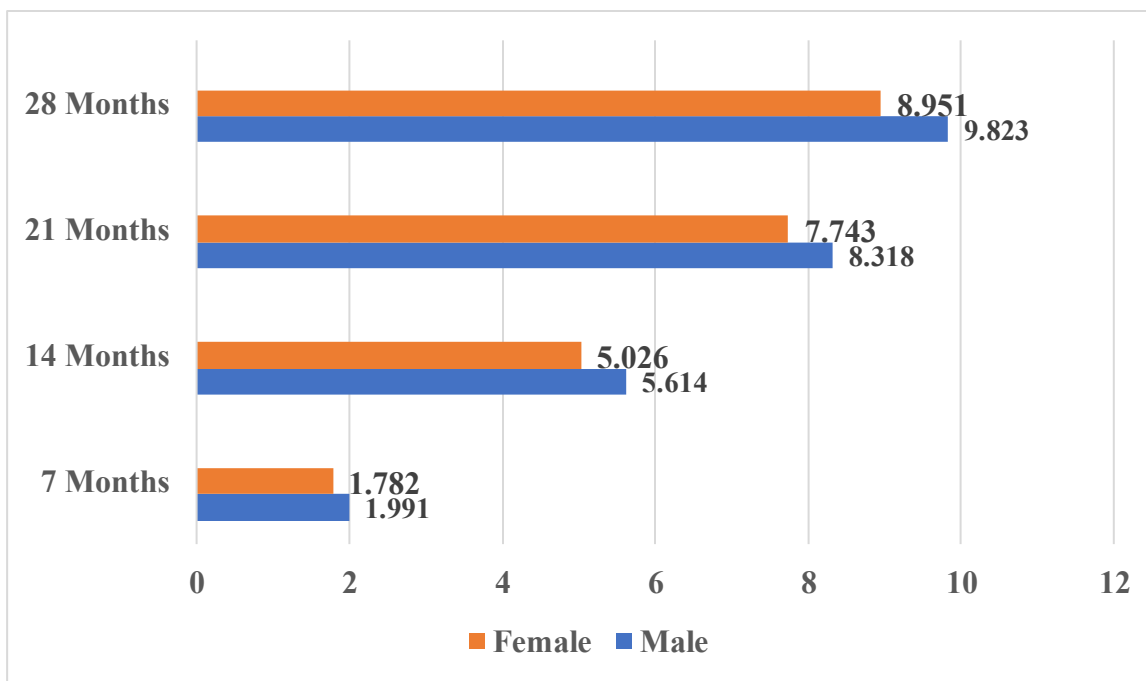


Fig. – 6: Age (months) and Concentration of Beta Amyloid Peptides (Ab42) in the Cerebro-Spinal-Fluid (C.S.F.) (Unit: picogram per milligram) in Male (n=25) and Female (n=25) Brown-rat, *Rattus norvegicus* (L).