EXPLORING THE CORRELATIONBETWEEN SERUM LEPTIN LEVELS AND OBESITY ASSOCIATED COGNITIVE DYSFUNCTIONIN THE BACKGROUND OF SYSTEMIC INFLAMMATION–AN OBSERVATIONAL STUDY

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KEYWORDS
LEPTIN, OBESITY, BMI, COGNITIVE DYSFUNCTION

ABSTRACT:
Previous studies have stated the correlation of leptin resistant obesity with cognitive dysfunction in severe cognitive dysfunctional disorders like Alzheimer's and Parkinson's but the correlation of leptin to mild cognitive dysfunction in obese individuals with no disorders is yet to be explored. The correlation of level of systemic inflammation with the level of cognitive decline is also to be explored.In this study we aimed to correlate the serum leptin levels with cognitive dysfunction in obese individuals with varying BMI levels and to correlate the systemic inflammatory marker levels with leptin. We collected blood samples to analyse serum leptin and systemic inflammatory markers and correlated them with BMI and cognitive functions using neuro - cognitive scale. We found a positive correlation between the metabolic parameters like BMI and leptin with systemic inflammatory markers indicating the pathophysiology behind neurodegeneration triggered by inflammation. We also found a negative correlation of cognitive state with BMI and leptin in obese individuals with no known neurodegenerative disorders

INTRODUCTION:

LEPTIN -A major obesityhormone derived from adipose tissue, acts via its receptor (LpR) – (JAKSTAT) predominantly in the hypothalamic nucleus to regulate energy balance and metabolism. Recent studies have shown its role in extra-hypothalamic targets like hippocampus. Leptinacts as acognitive enhancer by increasing the hippocampal dependant learning. Alteration in neuronal cellular morphology and glutamate receptor - dependent synaptic plasticity alters the hippocampal dependant learning(1).

EFFECT OF LEPTIN RESISTANCE ON HYPOTHALAMIC NEUROGENESIS: In High-fat diet induced obesity, hypoxia in the growing adipocyte tissues activates a pro inflammatory state.This increases the release of various adipokines including leptin, TNF- α (Tumor Necrosis Factor - alpha), IL-6 (Interleukin – 6) and CRP (C-reactive protein).In hypothalamus and hippocampusthis inflammatory change causes astrogliosis and microglial activation thus reducing the receptor sensitivity to leptin. Leptinresistance leads to reduced levels of proopiomelanocortin (POMC) neurons activation causing disturbance in energy balance. By altering the glutamate receptor mediated response leptin resistance leads to decreased hippocampal dependant synaptic learning. [2,3,4]

Study on AD (Alzheimer's disease) patients has shown significant upregulation of leptin levels in CSF(Cerebro Spinal Fluid) and hippocampal tissue. Additionally, astudy on Parkinson patients has shown a decrease in leptin receptor mRNAs and localization of receptor proteins to neurofibrillary tangles, suggesting that the leptin resistance in hippocampal tissue is associated with the disease.[5]

Previous studies have stated the correlation of leptin resistant obesity with cognitive dysfunction in severe cognitive dysfunctional disorders like Alzheimer's and Parkinson's but the correlation of leptin to mild cognitive dysfunction in obese individuals with no disorders is yet to be explored. The correlation of level of systemic inflammation with the level of cognitive decline is also to be explored. In this study we aim to correlate the serum leptin levels with cognitive dysfunction in obese individuals with varying BMI levels and to correlate the systemic inflammatory marker levels with leptin.

METHODOLOGY AND MATERIALS:

This is across-sectional studywith a sample size of 118, calculated usingOpenEpi sample size calculator (version 3) withreference data from RBANS (Repeatable battery for assessment of

neuropsychological status). The study was conducted over 6 months duration with individuals between 18 to 35 years attending the out-patient department of Thanjavur medical college.
Those with Age more than 35 year were excluded from the study to avoid aging related dementia changes,additionally we excluded those with known neurodegenerative disorders causing dementia like parkinsonism disease, Alzheimer's disease, uncontrolled hypertension (with risk of vascular dementia), type 2 diabetic mellitus (associated with microvascular brain injury), hypothyroidism, and Cushing's syndrome. Individuals who are on medications which affects their cognitive status where also excluded.Ethical approval was obtained from The Institutional Ethical Committee (C.N:684/2021), Thanjavur Medical College, Thanjavur, Tamil Nadu.
After obtaining written and informed consent, height was measured using a stadiometer, weight using digital weighing machine, BMI using Quetelet index weight (Kg)/Height (m^2) and subjects were groupedaccording to WHO Classification of BMI after Modifying for Asian population) as[6]

BMI LEVELS	**OBESITY CATEGORY**
18.5 - 22.9	Normal weight
23.0 - 24.9	Overweight
25.0 - 29.9	Obese class I
>30	Obese class II

Everywhere in this study BMI is being used as an indicator of general obesity.
We collected a 5μl serum sample after 8 hours of overnight fasting, from which serum leptin levels were analysed by solid phase sandwich ELISA – human leptin (Proquantum immunoassay kit - Anhui Anke Biotechnology (Group) Co., Ltd., china). The normalrange (according to assay kit reference) was 2.0 - 7.2 ng/ml for males and 3.7-11.1 ng/ml for females. Inflammatory marker with their respective assay kit references as TNF alpha (undetectable to <1pg/ml), IL-6 (<5pg/ml) and CRP (<0.9mg/dl) were analysed by human ELISA kit (by ThermoFisher scientific).
Cognitive status of the patient was analysed using MMSE (Mini Mental State Examination) scale, which has a total score of 30. The subdomains include Orientation (score: 1-10), Registration (score: 1-3), attention and calculation (score: 1-5), recall (score: 1-3)andlanguage (score: 1-9). Accordinglydecline in cognitive status was graded as >27(normal), 18-26 (mild dementia), 10-18 (moderate dementia), and<10 (severe dementia). [7]
Descriptive data are presented as mean and standard deviation. Statistical analysis was done usingPearson correlation coefficientto compare and correlate the variables. Everywhere p values < 0.05 was considered tobe statistically significant. All statistical analyses were done using SPSS software version 16.0for Windows (SPSS Inc.,).

RESULTS:

TABLE 1: Descriptive analysis

PARAMETER	N	MIN	MAX	MEAN	S. D
AGE	112	18	35	28.21	4.90
BMI	112	18.64	35.52	29.10	2.80
Serum LEPTIN	112	5.60	39.2	24.7	8.32
Cognitive score	112	18	30	28.2	2.9
TNF-α	112	1.53	2.59	1.91	0.37
IL-6	112	5.43	8.62	6.83	0.72
CRP	112	0.4	2.9	1.70	0.6

TABLE 1 Shows the descriptive statistics of age, BMI, serum leptin concentration, cognitive score and inflammatory marker levels in the study population

TABLE 2: Correlation analysis of BMI and leptin

CATEGORY	BMI* (kg/m^2)	LEPTIN (ng/ml)	CORRELATION (r)	pValue
OVERWEIGHT	24.2	15.8	+ 0.978	0.001*
OBESE 1	27.66	27.7	+ 0.989	0.001*
OBESE 2	33.2	35.73	+ 0.889	0.001*

TABLE 2 shows the strong positive correlation of BMI levels with serum leptin concentration

	Leptin (ng/ml)	Cognitive scores	r	pValue
OVERWEIGHT	15.8	27.80	-0.867	0.001*
OBESE 1	27.7	24.7	-0.872	0.001*
OBESE 2	35.73	21.6	-0.685	0.001*

TABLE 3: Correlation analysis of BMI, leptin and cognitive scores

TABLE 3 shows the negative correlation of metabolic parameters with cognitive scores

TABLE 4: Correlation analysis of leptin and inflammatory markers

Parameters	Mean	R	pValue
BMI x leptin	29.1 ± 2.8	0.857	0.005*
TNF-α x leptin	1.91 ± 0.37	0.977	0.005*
IL-6 x leptin	6.83 ± 0.72	0.938	0.005*
CRP x leptin	1.70 ± 0.60	0.834	0.005*

TABLE 4 shows the strong positivecorrelation of metabolic parameters with pre-inflammatory marker levels. (TNF-α: Tumor Necrosis Factor alpha; IL-6: Interleukin 6; CRP: C-Reactive Protein)

DISCUSSION:

Previous studies have stated the correlation of leptin resistant obesity with cognitive dysfunction in severe cognitive dysfunctional disorders like Alzheimer's and Parkinson's but the correlation of leptin to mild cognitive dysfunction in obese individuals with no disorders is yet to be explored. The correlation of level of systemic inflammation with the level of cognitive decline is also to be explored. In this study we aim to correlate the serum leptin levels with cognitive dysfunction in obese individuals with varying BMI levels and to correlate the systemic inflammatory marker levels with leptin.

With decades of research the role of leptin in regulating hunger and satiety is well established. Presently, with studies establishing the dynamic role of leptin in neuroprotective processes, researchers are exploring the precise mechanisms linking leptinto cognitive decline in neurodegenerative conditions like Alzheimer's and Parkinson's disease.[1]But still the exact role to leptin in memory loss remains explained. This studytried to explore the correlation between leptin and memory loss with relation to obesity and its associated systemic inflammation.

Leptin a major obesityhormone, derived from adipose tissueas a product of obese (ob) genemodulates food intake, body mass, and reproductive function via its receptor (LEP-R)in hypothalamic nucleus, it also plays a role in foetal growth, proinflammatory immune responses, angiogenesis and lipolysis. Studies in human and animal models have shown,leptin resistance characterized by elevated serum leptin levels leads to reduced satiety, over-consumption of nutrients, and increased total body mass[8,9,10,11].Studies on mice models also have shown that proteolytic cleavage of leptin receptor proteins leads to cell function loss and development of metabolic syndrome[12,13]. Rodent models to study on type 2 diabetes have also shown abnormal leptin signalling is related to systemic effects seen in absence of appetite control.[14]Studies on high-fat diet in normal and transgenic mice have also shown that leptin levels correlated with the amount of body lipid, but even with increased leptin levels their caloric intake did not come down suggesting resistance after a set point body weight. [15] our study also has shown a positive correlation between leptin and increasing BMI levels.

Studiesin various mouse models and some human studies relate BMI to severity of dementia in Alzheimer's disease(AD) patients. A study from Sweden shows that a two-fold higher incidence of dementia was associated with high midlife central adiposity in women . [16,17] Study from Canada on obesity induced cognitive impairment have proposed that excessive fat deposition in obeseindividuals is associated with adipose tissue dysfunction and activation of low degree inflammation by increasing the adipokines and proinflammatory cytokine levels thereby causing structural and functional endothelial dysfunctionaltering the cerebral microvasculature, which promotes cognitive impairment.[1]An 18 year follow up study in an urban population of Sweden among overweight persons showed a positive relationship between BMI and development of dementia.[18]But the mechanism by which obesity alters cognition is not studied extensively. Our study also showed a decline in cognitive functions with increasing BMI levels.

Based on study by Tartaglia et alwho isolated leptin receptor (Ob-R) from mouse choroid plexus using cloning techniques, [19] further studieson brain sections from leptin receptor - deficient mouse models like Zucker rats and db/dbobese mouse showed leptin receptors not only in hypothalamus but also in extra hypothalamic areas. [20,21]Studies from other sources have also show the presence of leptin receptors in cognitive brain areas. [22,23]In hippocampus leptin aids in long-term potentiation (LTP) and long-term depression (LTD) of spatial-memory.[4] Study in mouse models states, hypertriglyceridemia in obese impairs leptin's ability to cross blood brain barrier affecting hippocampal long-term potentiation.[24] A studyon AD patients has shown a deficiency of plasma leptin as an indicator of potential CNS leptin deficiency and leptin replacement therapy in such patients have shown improvement in cognitive functions. [22] Post autopsy tissue samples have shown elevated leptin levels in CSF and hippocampal tissue with decreased the level of leptin receptor mRNA in AD patients. [5]A study analysing the possible role of leptin in altering the hippocampal functions found modulation of NMDA (N-Methyl D-Aspartate) receptor to be responsible for the hippocampal excitatory synaptic long-term potentiation. [24]Study on grey matter volume in comparison to leptin levels showed increased levels of leptin didn't improve the apoptotic neuronal death probably suggesting impact of leptin resistance in neuronal dysfunction [25]in our study we also see a positive correlation of leptin resistance as indicated by elevated serum leptin levels with decreasing cognitive scores. Which might be a linking bridge between obesity and cognitive dysfunction.But study from Netherlands using serum samples from AD patients showed no correlation between leptin and cognitive decline however states leptin alteration could be important in the preclinical stage of initial disease development with no further effects at the clinical stage of the disease.[26]Mice models trained for T-maze foot shock avoidance showed improved memory retention with leptin injections immediately after the tests showing the role of leptin hippocampal related memory processing. [27] Study on leptin treated mice showed improved cognitive performance in new object recognition and fear conditioning tests by reducing the amyloid load within hippocampal areas. [28, 29, 30] .In our study also we see an elevated leptin level is associated with declining cognitive scores in relation to increasing BMI levels

LIMITATION:
Study can further be done with various cognitive scales to enhance the strength of association. The correlation of systemic inflammation with neuroinflammation with the help of spectrometric studies would further broaden our views on the topic.
CONCLUSION:
Previous studies have stated the correlation of leptin resistant obesity with cognitive dysfunction in severe cognitive dysfunctional disorders like Alzheimer's and Parkinson's but the correlation of leptin to mild cognitive dysfunction in obese individuals with no disorders is yet to be explored. The correlation of level of systemic inflammation with the level of cognitive decline is also to be explored. In this study we aimed to correlate the serum leptin levels with cognitive dysfunction in obese individuals with varying BMI levels and to correlate the systemic inflammatory marker levels with leptin. We found a positive correlation between the metabolic parameter like BMI and leptin and systemic inflammatory markers indicating the pathophysiology behind neurodegeneration triggered by inflammation. We also found a negative correlation of cognitive state with BMI and leptin in obese individuals with no known neurodegenerative disorders.
Author contribution:
DR. S. D. DHEEBIKA – Principal investigator and formulating of the manuscript
DR. SANJAY ANDREW RAJARATNAM – Reviewing and editing of the manuscript
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Data availability statement:
The data are not publicly available as it contains sensitive information of the participants.

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