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ОРИГИНАЛЬНОЕ ИССЛЕДОВАНИЕ

Neurophysiological cognitive assessment and its association with neutrophil to lymphocyte ratio

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Abstract. Relevance. Cognition is an important physiological and higher mental functions in human being. There are various studies showing that inflammatory condition could negatively affect fronto temporal cognitive abilities such as memory, attention and executive functions. A non-invasive test P300 a component of Auditory events related potentials and Mini mental state examination (MMSE), a questionnaire based test reflect cognitive function, and haematological parameter neutrophil/lymphocyte ratio (NLR) is a convenient parameter of systemic inflammation. *Aim* of present study was to assess the cognitive function assessment by P-300, MMSE and academic performance and find an association with neutrophil to lymphocyte ratio in first year medical students. *Materials and Methods.* This was an observational study conducted on 79 first year medical students of age group 18–25 years in the department of physiology RUHS College of medical sciences Jaipur. For cognitive assessment non-invasive test P300, MMSE and academic performance was recorded. A haematological parameter NLR was calculated by dividing the absolute neutrophil count with the absolute lymphocyte count. To find an association statistical analysis was done by MEDCALC 16.4 version software. *Results and Discussion.* The association between P-300 amplitude and latency and MMSE with NLR was found non-significant. Marks have a significant positive correlation with NLR (0.015). *Conclusion.* In the present study neurocognitive function test P-300 and MMSE found non-significant association with inflammatory marker NLR although academic performance (marks) have a significant positive correlation with NLR.

Key words: cognition, event related potential, neurocognitive function, neutrophil / lymphocyte ratio, mini mental state examination

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materials, writing text; Sudhanshu Kacker concept and design of research, writing text; Naina Jangid- writing text. All authors have made significant contributions to the development concepts, research and manuscript preparation, read and approved final version before publication.

Conflict of interest statement. The authors declare no conflict of interest.

Ethics approval. Prior to starting the study institutional ethical committee clearance was taken. (letter no.RUHS-CMS/ETHICS Comm./2019/09 dated 12–3–19, No.EC/P-54.1/2018).

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Consent for publication. Written voluntary consent was obtained from the participants for the investigation and publication of relevant medical information according to WMA Declaration of Helsinki — Ethical Principles for Medical Research Involving Human Subjects, 2013.

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Introduction

Cognition is a broad concept which includes: perception, attention, working memory, reasoning, problem solving, language skills and decision making [1]. It represents important physiological and higher mental functions in human being. There are various evidence has linked inflammation (an immune response to injury, pathogens, irritants, oxidative stress) to cognitive decline and risk of dementia [2]. Normally, inflammation is a protective response that facilitates the healing process; however, prolonged inflammation can cause tissue damage.

Cognition test can be assessed by a non-invasive test P300 a component of Auditory events related potentials (AERP) which reflects attention and memory process and give characteristic features of information processing in terms of latency and amplitude in the central nervous system. Recent neurophysiological studies investigated individual variation in different cognitive processes including information processing, working memory, and intelligence by measuring event-related potentials (ERPs) [3, 4].

The MMSE is a brief cognitive screening questionnaire based instrument frequently used to evaluate cognitive function, has related brain structures that are responsible for this function.

It has been well documented that inflammatory processes may cause mental illness by brain degeneration. A haematological parameter neutrophil/lymphocyte ratio (NLR) is a convenient parameter of systemic inflammation [5] that can be easily calculated from white blood cell essay, the ratio of absolute neutrophil count to the absolute lymphocyte count, very easy, inexpensive, reliable, objective and convenient method. Regulation of immuno-inflammatory control is one of the relevant processes involved in the pathogenesis of neurodegenerative disorders. There are various studies showing that inflammatory condition could negatively affect fronto-temporal cognitive abilities such as memory, attention and executive functions [6]. It was shown that NLR have diagnostic and predictive value in diseases like Alzheimer's disease [7]. Dementia, Schizophrenia etc. and may guide the treatment of cognitive dysfunction. In our study we tried to find the association of cognitive status with NLR. There are several studies that have shown an association between the increase in NLR and psychopathologies. This has been observed for patients with schizoaffective disorder and schizophrenia [8], attention deficit hyperactivity disorder [9], obsessive compulsive disorder [10], hypomania in bipolar mood disorder [11], and major depressive disorders [12, 13]. Aim of present study was to assess the cognitive

function assessment by P-300, MMSE and academic performance and find an association with neutrophil to lymphocyte ratio in first year medical students.

Materials and methods

The study was an observational study conducted on first year students of RUHS-CMS in Department of Physiology, RUHS-CMS, JAIPUR after taking permission from the institutional ethics committee (letter no. RUHS-CMS/ETHICS Comm./2019/09 dated 12–3–19, No.EC/P-54.1/2018). The age group of students were 18–25 years. Written consent was taken before enrolling the students and participants information sheet was given to them. 100 students were enrolled but only 79 were continued in study. The duration of study was six months from March 2019 to September 2019. Subjects with hearing and visual defects, neurological, psychiatric, haematological disorders and genetic disorders and with chronic diseases like diabetes, hypertension, gastrointestinal diseases (GI ulcers, haemorrhoids) were excluded during subject selection time. General physical examination was done and histories were taken (personal, family history of HT, DM, neurological illness, socioeconomic status, dietary history etc.).

Following parameters were taken for data collection:

Anthropometric parameters — height, weight, body mass index (BMI) etc. were taken using the standard protocol of Weiner and Lourie. Haematological investigations — blood sample was collected with the help of technician using aseptic technique uniformly from all the subjects and sent to the laboratory for estimation of complete blood counts (CBC). The NLR ratio be calculated by dividing the absolute neutrophil count with the absolute lymphocyte count: [neutrophil count]/[lymphocyte count].

Neurological investigation for measurement of cognitive function:

A. Auditory cognitive evoked potential (P300)—by using (NCV/EMG machine — make-clarity); the P300 was measured using auditory odd ball paradigm. The stimulus that was being given in order to evoke an endogenous potential is auditory in nature. In a dimly lit room the subject was asked to sit on a chair, comfortably with

closed eyes and remain awake and alert. The subject was instructed in prior to restrict the eyeball movement in order to avoid any electro — ocular artefacts or contamination. The subject was instructed to keep a mental count of the numbers of target stimuli by raising the finger.

Electrodes were fixed on the scalp with the help of a conductive paste. Electrodes were placed according to 10–20 international system of EEG electrode placement. Active electrode was placed at Cz. Reference electrodes were linked to right and left mastoid (A1 and A2). The ground electrode was placed at Fpz. Two tones were used as stimulus, a frequent low pitched tone and a rare relatively high pitched tone. Subject was asked to attentively count the number of rare stimuli and ignore the frequent stimuli. As soon as a novelty stimulus i.e. a rare stimulus was attended by the subject, it results in recording of an evoked potential. Approximately two traces were taken per recording. N1 and P1 waves were recorded in response to the frequent stimulus, while P3 or P300 was a large positive deflection of wave captured on attending the rare stimuli. Responses were averaged until minimum 25 stimuli, 100 frequent are stimuli given and amplitude and latency was recorded as data [14].

B. Mini mental state examination (MMSE): the MMSE is a brief cognitive screening instrument frequently used to evaluate cognitive disorders. This comprises of 11 questions and assesses 6 cognitive functional areas: awareness, focus, immediate memory, short-term recall, vocabulary, and ability to follow basic verbal and written commands. The assessment is developed as a standardised instrument which offers a total score that allows the patient to be put on a cognitive functional scale. Each MMSE-evaluated cognitive function has related brain structures that are responsible for this function. The subject maximum score is 30 and scores <24 are associated with cognitive impairment [15].

C. Marks of 1ST M.B.B.S. university exam were maintained as data to assess cognition.

Statistical analysis was performed using unpaired ‘t’ test; $p < 0.05$ (significant). The study findings are reported as mean \pm S.D. Statistical analysis was done by MEDCALC 16.4 version software. In this p-value if lower than conventional 5 % ($p < 0.05$) the coefficient

is called statistically significant and the 95 % confidence interval for the correlation coefficient shows true correlation coefficient.

Results and discussion

There were 100 students enrolled. All the required parameters were taken as per approval condition of the ethical committee and research protocol. Only 79 out of 100 students had been continued in the study. There were 39 male and 40 were females.

Table 1 showed the Mean \pm SD of age, BMI, NLR, P-300 latency & amplitude and MMSE, marks of 79 subjects.

Table 1

Baseline characteristics and laboratory data of the studied groups

	N	Mean	SD	Median	Minimum	Maximum
Age	79	19.13	0.98	19	18	24
BMI	79	24.44	14.90	22	15.1	150
NLR	79	1.90	0.70	1.71	0.7	5
P-300 Avg (amp)(μ v)	79	2.99	2.00	2.7	0.1	10
P300 Avg latency (ms)	79	261.70	39.50	264.4	184.4	345
MMSE	79	26.41	1.93	27	21	30
Marks	79	366.10	43.25	374	263	443

Note: BMI – body mass index; NLR – neutrophil/lymphocyte ratio; MMSE – mini mental state examination.

Table 2 showed that the male and female mean age was (19.39 \pm 1.09 years, 18.88 \pm 0.79 years) respectively and found a significant difference (p value 0.020). The male and female having mean BMI (23.15 \pm 4.63, 25.71 \pm 20.48) respectively and found non-significant differences (p value 0.449). The male and female mean NLR value was (1.72 \pm 0.58, 2.07 \pm 0.76) respectively and found a significant difference (p value 0.023).

For cognitive assessment the P-300 amplitude and latency were measured. The mean value of P-300 amplitude in male and female was found (2.98 \pm 1.95 μ v) and (3.00 \pm 2.06 μ v) respectively with a non-significant difference (p value 0.972). The mean value of P-300 latency in male was (259.63 \pm 37.17ms), and in female (263.71 \pm 42.02 ms) and found non-significant difference (p value 0.649).

Mean MMSE score in male and female was (26.08 \pm 2.08) and (26.73 \pm 1.74) respectively and non-significant difference was found (p value 0.137).

Mean university marks in male and female was (344.62 \pm 39.0) and (387.05 \pm 36.64) respectively and found significant difference (p value <0.001).

Table 2

Comparison of variables between two groups (male and female)

Variables	Sex	N	Mean	SD	Median	Min.	Max.	'p' value*
Age	Male	39	19.39	1.09	19	18	24	0.020
	Female	40	18.88	0.79	19	18	20	
BMI	Male	39	23.15	4.63	22.2	15.1	38.6	0.449
	Female	40	25.71	20.48	21.9	17.1	150	
NLR	Male	39	1.72	0.58	1.67	0.7	3.4	0.023
	Female	40	2.07	0.76	1.9	0.97	5	
Avg (amp) (μ v)	Male	39	2.98	1.95	2.9	0.1	10	0.972
	Female	40	3.00	2.06	2.35	0.6	7.9	
P3 avg (ms)	Male	39	259.63	37.17	264.4	184.4	333.15	0.649
	Female	40	263.71	42.02	264.1	186.25	345	
MMSE	Male	39	26.08	2.08	26	21	30	0.137
	Female	40	26.73	1.74	27	22	29	
Marks	Male	39	344.62	39.07	345	275	427	<0.001
	Female	40	387.05	36.64	393	263	443	

Note: BMI – body mass index; NLR – neutrophil/lymphocyte ratio; MMSE – mini mental state examination; * – unpaired 't' test; p < 0.05 (significant).

Table 3 showed that the correlation was done of cognitive function assessment parameters with age, BMI and NLR. The P-300 Amplitude and Latency have no significant correlation with age, BMI and NLR. MMSE and BMI found significant negative correlation (0.010). Academic performance (marks) have significant negative correlation with age and BMI (0.0028, 0.0132) respectively and significant positive correlation with NLR (0.015).

In the present study we tried to find the association of cognitive status with inflammatory marker neutrophil lymphocyte ratio. Cognitive assessment done by neurophysiological tests-event related potential –300, MMSE questionnaire based test and on the basis of academic performance in first MBBS university exam. There were many studies showing that inflammatory condition could negatively affect fronto temporal cognitive abilities such as memory, attention and executive functions [16]. Neutrophil lymphocyte ratio (NLR) regarded as a marker of the body's immune response and considered as a rapid

Table 3

**Correlation of dependent variables with other studied variables
in studied subjects**

Dependent Variables		Age	BMI	NLR
P-300 Amplitude Avg (μ v)	Sample size	79	79	79
	Correlation coefficient r	-0.00573	-0.08116	-0.03868
	Significance level	0.960	0.4771	0.735
	95 % CI for r	-0.2266–0.2157	-0.2969–0.1425	-0.2576–0.1840
P-300 Latency avg (ms)	Sample size	79	79	79
	Correlation coefficient r	0.1816	-0.01048	0.03712
	Significance level	0.1092	0.927	0.7453
	95 % CI for r	-0.04114–0.3872	-0.2311–0.2111	-0.1855–0.2561
MMSE	Sample size	79	79	79
	Correlation coefficient r	-0.1495	-0.2884	-0.1085
	Significance level	0.1885	0.010	0.3413
	95 % CI for r	-0.3588–0.07405	-0.4790 – -0.07185	-0.3219–0.1154
Marks	Sample size	79	79	79
	Correlation coefficient r	-0.3316	-0.2779	0.2729
	Significance level	0.0028	0.0132	0.015
	95 % CI for r	-0.5149 – -0.1192	-0.4701 – -0.06048	0.05511–0.4659

Note: BMI – body mass index; NLR – neutrophil/lymphocyte ratio; MMSE – mini mental state examination.

and simple parameter to indicate the systemic inflammation and stress, that causing increased permeability of the blood-brain barrier, exposing the brain to toxins, reactive oxygen species originating in the systemic circulation and may leads to the process of oxidization and inflammation and eventually results in causing neurodegeneration [17, 18]. NLR is the ratio of absolute neutrophil count to the absolute lymphocyte count. In recent years, an increasing number of studies have focused on NLR. Prognostic role of NLR is continually being investigated and emerging as a robust predictor of deleterious outcomes in many diseases. The potential role of NLR in Alzheimer's disease (AD) was first investigated by Kuyumcu et al. (2012) who found that NLR was significantly higher in AD patients compared to controls [19]. The relationship of NLR with cognitive impairment has been suggested by liu et al (2020) and found higher NLR in patients with cognitive decline [20]. Neutrophils are the first line of immune defence: they exhibit phagocytic and apoptotic action through the secretion of various inflammatory

factors, in particular, cytokines [21]. Inflammation triggered by cytokines can induce further inflammation due to cell dysfunction and to oxidative stress. On the other side lymphocytes are specific inflammatory mediators, with a regulatory or protective function; low lymphocyte counts reflect poor general health and physiologic stress [22], Mechanism of how systemic inflammation and increased NLR result in cognitive impairment is unclear. Inflammation characterized by increased neutrophils and decreased lymphocytes can reduce plaque stability and promote atherosclerosis, which may increase the risk of delirium through micro infarcts.

The cognitive function tests we have done was P300 and it may have multiple intracerebral generators, with the hippocampus and various association areas of the neocortex all contributing to the scalp-recorded potential and represent the transfer of information to consciousness, a process that involves many different regions of the brain.

Patrice Forget, Céline Khalifa et al (2017), found in their study normal NLR value in adults is 0.78 to 3.53 [23]. In our study mean NLR Value in male and female is 1.72 and 2.07 respectively and found significant difference was found between male and females (P value 0.023). In our study we tried to find the relationship of neurophysiological test of cognition with NLR. The subjects participated was healthy students and we found no significant association between P-300 and MMSE and marks with NLR.

Study done by Jaime ramos cejudo et al in 2021 found an association between NLR and risk of subsequent dementia in the Framingham heart study [24].

Hadi J. Halazun et al. studied the neutrophil-lymphocyte ratio as a predictor of cognitive dysfunction in carotid endarterectomy patients and found that the patients with cognitive dysfunction had significantly higher NLR than those without cognitive dysfunction [25]. Kalelioglu et al. studied the neutrophil and platelet to lymphocyte ratios in people with subjects of mild cognitive impairment and early Alzheimer's disease [26]. Gorelick P.B. et al. show the role of inflammation in cognitive impairment [27].

Conclusion

In the present study cognitive function test P300 and MMSE were found no association with NLR. Marks have a significant positive correlation with NLR. Correlation with in dependable parameter shows that MMSE have significant association with BMI, and marks have significant association with age, gender and BMI. In the present study neurocognitive function test P-300 and MMSE found non-significant association with inflammatory marker NLR although academic performances (marks) have a significant positive correlation with NLR.

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Нейрофизиологическая оценка когнитивных функций и связь с соотношением нейтрофилов и лимфоцитов

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Аннотация. *Актуальность.* Познание является важной физиологической и высшей психической функцией человека. Существуют различные исследования, показывающие, что воспалительное состояние может негативно повлиять на лобно-височные когнитивные способности, такие как память, внимание и исполнительные функции. Неинвазивный тест P300, компонент потенциалов, связанных со слуховыми событиями и минимальной оценкой психического состояния, а также тест на основе опросника отражают когнитивную функцию, а гематологический параметр соотношения нейтрофилов/лимфоцитов является удобным параметром системного воспаления. Цель настоящего исследования состояла в том, чтобы оценить степень когнитивных функций по опроснику P-300, минимальную оценку психического состояния и академической успеваемости и найти связь с соотношением нейтрофилов и лимфоцитов у студентов-медиков первого курса. *Материалы и методы.* Это было наблюдательное исследование, проведенное на 79 первокурсниках-медиках возрастной группы 18–25 лет на кафедре физиологии RUHS колледжа медицинских наук г. Джайпура. Для оценки когнитивных функций регистрировали ответы на неинвазивный тест P300, проводили минимальную оценку психического состояния и анализировали успеваемость. Гематологический параметр соотношения нейтрофилов/лимфоцитов рассчитывали путем деления абсолютного количества нейтрофилов на абсолютное количество лимфоцитов. Для обнаружения ассоциации был проведен статистический анализ с помощью программного обеспечения MEDCALC версии 16.4. *Результаты и обсуждение.* Связь между амплитудой P-300 и минимальной оценкой психического состояния с соотношением нейтрофилов/лимфоцитов оказалась незначительной. Успеваемость имеет значительную положительную корреляцию с соотношением нейтрофилов/лимфоцитов (0,015). *Выводы.* В настоящем исследовании

тест нейрокогнитивных функций Р-300 и минимальной оценки психического состояния обнаружили незначительную связь с воспалительным маркером соотношения нейтрофилов/лимфоцитов, хотя академическая успеваемость (оценки) имеет значительную положительную корреляцию с соотношением нейтрофилов/лимфоцитов.

Ключевые слова: познание, событийный потенциал, нейрокогнитивная функция, соотношение нейтрофилов и лимфоцитов, мини-обследование психического состояния

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