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S.N	Publication type	Publication title	Author name	Journal name	Year
1.	Original article	The Impact of COVID-19 on Auditory and Visual Choice Reaction Time of Non-hospitalized Patients: An Observational Study.	Seetharam Kausikan, Sengottaiyan Anu, Mohanraj Saravanan, Thulasiraman Devisri, Kanagamuthu Rekha, Rajpathy John	International Journal of Scientific Research in Dental and Medical Sciences	2022
2.	Original article	Effect of Mint-flavored Chewing Gum in Observing Changes in Cognitive Function while assessing Test Performance	S Anu, K Jeyashree, SN Vishnuvarthini, N Prasanna Venkatesh, J Vijay Anto	Journal of Clinical and Diagnostic Research	2022
3.	Original article	Utility of biochemical markers in predicting severe Covid-19: experience from a tertiary hospital in south India.	Mamatha T. Shenoy, Pradipta Kumar Mohanty, K. Suganthy, Jeya Kumar Manavalan, Hariharan Alexander	The journal of the International Federation of clinical chemistry and laboratory medicine	2022
4.	Original article	Evaluation Of Association Of Serum Leptin With Chronic Complications Of Diabetes And Glycemic Control	Hariharan A, Sumathi S and Asmathulla S	Journal of Cardiovascular Disease Research	2022
5.	Original article	Evaluation of Haematological Findings in Tuberculosis Patient of Madurai- An Cross Sectional Study	Niranjan Prabhakar, C. Ramesh, Sriandaal Venkateshvaran	International Journal of Medical Science and Current Research	2022

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6.	Original article	Clinico-radiological profile of bronchiectasis patients an observational study	Niranjan Prabhakar , V.Sriandaal , Basanth Harazika	International Journal of Scientific Development and Research	2022
7.	Original article	Effect of Mint Flavoured Chewing Gum in Observing Changes in Cognitive Function while Assessing Test Performance- An Interventional Study	S Anu, K Jeyashree, SN Vishnuvarthini, N Prasanna Venkatesh, J Vijay Anto	Journal of Clinical and Diagnostic Research	2022

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The Impact of COVID-19 on Auditory and Visual Choice Reaction Time of Non-hospitalized Patients: An Observational Study

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ABSTRACT

Background and aim: COVID-19 is a multi-system infectious disease. There is accumulating evidence showing the damage caused by the virus on a nervous system other than the lungs. It is still unknown whether this Central nervous system (CNS) complications are reversible. Reaction time indicates neuronal activity, and an increase in RT denotes defective neural function. Hence the present study was done to measure the impact of COVID-19 on auditory and visual reaction time during COVID-19 disease, four and eight weeks after recovery.

Material and methods: ART and VRT were measured using discriminatory and choice reaction time apparatus. Results were analyzed using an unpaired t-test. The study involved 86 participants of both genders aged 21-40. Forty-six were acute, mild COVID-19 positive patients and 40 were healthy (control) subjects.

Results: During COVID-19 disease, a statistically significant increase in visual reaction time (VRT) values for both green and red colours ($p<0.001$) and ART ($p<0.001$) were observed in the study group when compared with the control group. After 4 weeks of recovery, a significant increase in VRT ($p<0.05$) for both the colours and ART ($p<0.05$) were observed compared with control, but the values were lesser when compared with during COVID-19 disease in the study group. After 8 weeks, no statistically significant difference ($p>0.2$) was observed between both groups. No gender difference was detected.

Conclusions: Increased RT values indicate that COVID-19 affects the nervous system. The decline in RT values after 4 weeks and normal values after 8 weeks of recovery shows improvement in nerve function.

1. Introduction

The damage caused by COVID-19 infection (SARS-CoV-2) on the lungs is quite known. However, studies show the extension of this virus beyond the respiratory system, including the cardiovascular system, gastrointestinal system, renal system, and nervous system. Per recent studies,36.4% of patients with COVID-19 (especially severely affected individuals) had neurological symptoms, posing a significant risk of morbidity and mortality.[1] The commonly reported symptoms include headache, disturbed consciousness, paresthesia, loss of smell, taste and vision, partial neuronal degeneration, and brain tissue edema.[2] The common neurological manifestations during and after COVID-19 include encephalopathy, encephalitis, meningitis, acute cerebrovascular disease, and Guillain-Barre syndrome.[3] Reaction time (RT) measures the quickness with which a response occurs. It is the interval between the presentation of the stimulus and the motor response. Reaction time needs an intact sensory system, cognitive processing, and motor performance.[4] The stimulus could be auditory, visual, tactile. Factors affecting reaction time include gender, age, physical fitness, level of fatigue, distraction, alcohol, personality type, limb used for the test, and biological rhythm. Three basic reaction time paradigms have been described: (1) simple reaction time has a single stimulus and a single predefined response, (2) recognition reaction time has several false stimuli mixed with one correct stimulus prompting the response, and (3) choice reaction time involves multiple stimuli and differing responses for each stimulus.[5] In the present study, Choice reaction time was used as it has high test-retest reliability and also activated more brain areas. Choice reaction time studied using visual inputs is known as visual choice reaction time (VRT), and auditory inputs are known as auditory choice reaction time.

RT response will be faster with an intact nervous system. Faster RT also indicates better cognitive functioning, including memory, verbal fluency, and intelligence.[6] RT has an important role in day-to-day activities, from responding to a doorbell, answering MCQs on the internet, driving. Damage to the nervous system can impair these daily activities. The accepted values

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for the simple reaction time are 180-200 ms for light stimuli and 140-160 ms for sound stimuli.[7] It is unknown whether this injury to the nervous system is transient or persistent. Assessing reaction time is an indirect, non-invasive and simple way of examining the integrity of central and peripheral nervous systems. Hence the present study aims to determine the effect of COVID-19 on audiovisual reaction time in affected individuals during illness and after one and two months of recovery.

Objectives:

1. To measure the impact of COVID-19 on auditory and visual reaction time during COVID-19 illness.

2. To measure the impact of COVID-19 on auditory and visual reaction time four and eight weeks after recovery from the disease.

3. To measure the auditory and visual reaction time in healthy non-COVID-19 subjects.

4. To compare and analyze the auditory and visual reaction time values during and after 4, 8 weeks of COVID-19 patients with the control.

2. Material and methods

This observational, cross-sectional study was done in the Department of Physiology of a private medical college and in a district hospital between July - October 2021 with a study population of 86, of which 46 were COVID-19 positive (study) patients and 40 were non-COVID-19, healthy (control) subjects. The study was done using a non-probability sampling technique. Volunteered patients from the department of General medicine were included in the study. The study was conducted after obtaining Institutional ethical clearance (IEC no: VMCIEC/88/2021). Informed written consent was obtained from each participant. The purpose of the study was explained to them in the language they could comprehend. The confidentiality of the participants was ensured, and they were informed that they could withdraw from participating in the study anytime. RT-PCR COVID-19 positive subjects in the age group between 20 to 40 years of both the genders and with BMI between 22.3-25.3 were included in the study. Only mild COVID cases (score 0-4) as categorized by the disease severity classification system for COVID-19, Republic of Korea (Bulletin of World Health Organization) were included in the study. This classification is based on the pulse rate, systolic blood pressure, respiratory rate, body temperature and level of consciousness.[8] According to this, moderate cases had a total score of 5–6, and severe and very severe cases had a score $\geqslant 7$.

For the control group, healthy subjects aged 20 to 40 years of both genders were included. Subjects with diabetes, visual defects, ENT disorders, cardiovascular and respiratory diseases, psychiatric disorders, neurological disorders, cataracts, on drugs, smoking, and alcoholism were excluded from the study. Baseline data on all participants were collected using a structured questionnaire. All the participants were instructed to refrain from caffeine, smoke for 12 hours and have an adequate sleep before the day of testing. Weight was measured with an electronic weighing scale (Doctor Beliram and sons, New Delhi) and height with a stadiometer. VRT for green and red light and ART was measured with the help of discriminatory and choice reaction time apparatus (Anand Agencies, Pune). The study was conducted between 10-12 pm every day to evade the effects of the circadian rhythm. The same COVID-19 patients were followed-up after 4 and 8 weeks of recovery.

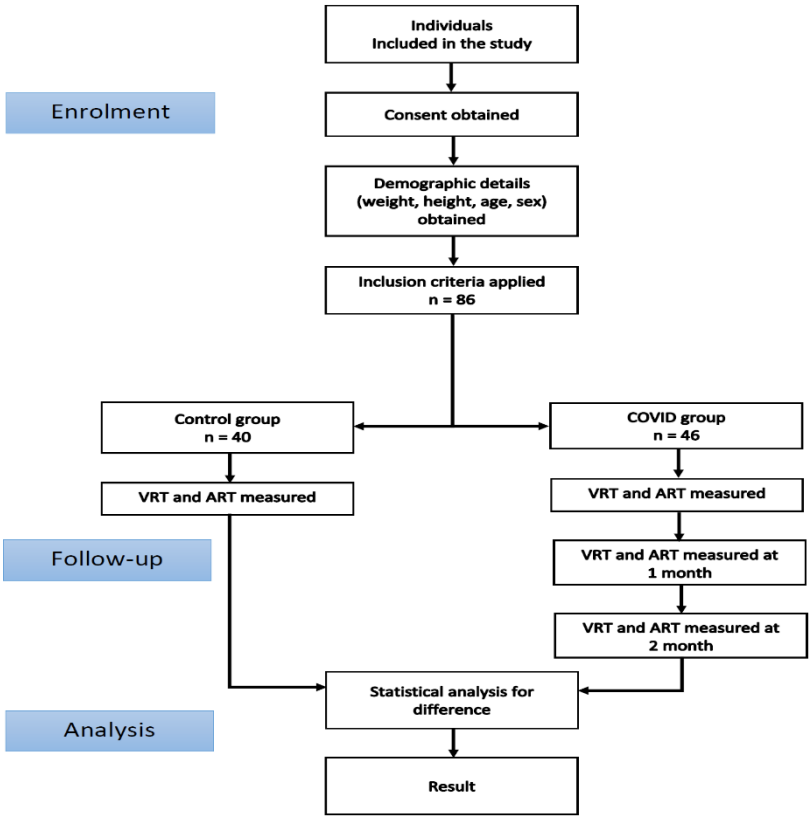

Fig. 1. Methodology flow chart.

Visual Reaction Time (VRT) and Auditory Reaction Time (ART) second were measured in the sitting posture in a quiet room with an accuracy of 0.001. The visual stimulus was provided by red and green light, and a beep sound provided the auditory stimulus. The subject was initially instructed about the complete procedure to record the baseline VRT for the green light. The subject should keep pressing the index finger of the right hand on the response button, and once he visualizes the stimulus, he immediately has to remove his finger. The response button terminated the clock counter and the value of VRT in milliseconds was displayed on the screen. Sufficient time was given for the participants to thoroughly get acquainted with the procedure. After the practice trial, three readings were taken once the patient felt comfortable, and the fastest response value was taken as the final reaction time. Baseline VRT for a red light was also recorded similarly. The same procedure was followed to collect the values in all COVID-19 subjects after 1 and 2 months. Proper COVID-19 guidelines were followed in handling COVID-19 patients, and the instruments used were sterilized every time before taking each value. For ART, after giving tone stimuli in the same apparatus, the subject was asked to release the key, which gave the ART value in milliseconds. Similarly, VRT and ART were recorded in control subjects also.

3. Results

Statistics analysis

The data was entered in MS Excel and analyzed using SPSS 24. The readings of AV reaction time during COVID-19 disease and after one and two months of recovery were analyzed and compared with the control group using an unpaired t-test. P-value less than 0.05 was the cut-off to determine statistical significance.

Table 1. Demographic and anthropometric details of the study (case) and control groups.

		Groups		P-value
		Control (40)	Case (46)	
Gender %	Male	12 (30%)	16 (35%)	0.402
	Female	28 (70%)	30 (65%)	
Age in years (mean±SD)		31.2±4.9	31.4±6.4	0.438
Range(years)		22-40	21-40	-----
BMI (mean±SD)		24.3±0.6	24.4±0.81	0.161
Range		22.6-25.2	22.3-25.3	-----

Table 2. Visual reaction time for green and red colour stimuli in the study versus control group during and after 4 and 8 weeks of recovery from COVID-19.

Mean ± SD	Control	COVID-19 0 week(during illness)	COVID-19 post four weeks	COVID-19 post eight weeks
Green	170.80±22.71	202.63±29.23	181.4±21.9	173.33±20.07
Red	161.92±21.79	201.46±26.08	181.93±21.52	164.50±17.55
P-value	-----	p<0.001*	p<0.05*	p>0.2

* Statistically significant

According to Table 2, during COVID disease, VRT values for green and red colour were significantly higher (p<0.001) in the study group than in the control group, indicating a delay in nerve conduction. After four weeks of recovery, there was a decrease in the mean VRT values of the study group, but a statistically significant increase (p<0.05) was observed compared with the control group. It shows that the nerve function has started improving. After eight weeks, complete recovery was noticed as no statistically significant difference (p>0.2) was observed between the study and the control group.

Table 3. Auditory reaction time in the study versus control group during and after 4 and 8 weeks of recovery from COVID-19.

Mean ± SD	Control	COVID-19 0 week(during illness)	COVID-19 post four weeks	COVID-19 post eight weeks
Tone stimulus	152.86±29.76	176.78±21.66	161.98±17.7	155.85±15.49
P-value	-----	p<0.001*	p<0.05*	p>0.2

* Statistically significant

According to Table 3, during COVID-19 disease, ART values were significantly higher ($p<0.001$) in the study group than in the control group indicating damage to the brain and the nervous system. After four weeks of recovery, though a statistically significant increase ($p<0.05$) was observed in the study group compared with the control group, mean ART values of the study group were lesser than during COVID-19 disease. This shows that the nerve function has started improving. After eight weeks, no statistically significant difference ($p>0.2$) was observed between the study and the control group showing marked improvement in nerve function.

Table 4. Gender differences in auditory and visual reaction time.

	Female VRT (Green)	Male VRT (Green)	Female VRT (Red)	Male VRT (Red)	Female ART	Male ART
Mean	200.3	207.1	200.1	204.0	178.1	174.3
SD	26.9	33.6	22.6	32.4	20.3	24.6
P-value	0.23		0.32		0.29	

According to Table 4, no statistically significant differences in ART and VRT values were observed between males and females.

4. Discussion

During COVID-19 disease, a statistically significant increase in VRT values for green and red colour ($p<0.001$) and ART ($p<0.001$) were observed in the study group when compared with the control group (Table 2). An increase in reaction time values indicates that more time is taken for conduction in sensory-motor pathways. This could be due to the nervous system damage caused by the COVID-19 disease. The present study results agree with previous studies on patients with brain diseases, where RT was found to be slower, especially in subjects with lesions in the left cerebral hemisphere.[9, 10] The slower reaction time indicates delayed neuronal activity.[11] Though the virus is detected in the CSF of infected patients, previous studies on COVID-19 had shown that the nervous system involvement could be due to widespread malfunction of the immune system and dysregulation of blood vessels rather than the direct entry of the virus into the brain.[12] Ischemic and demyelinating changes are seen in the cerebrum, cerebellum and hippocampus neurons in COVID-19 patients.[13] The presence of myelin sheath helps in faster conduction of impulses from one node to another node, as myelin insulates the axon and assembles voltage-gated sodium channels in the nodes of Ranvier. Demyelination slows down the conduction velocity along sensory and motor nerves prolonging RT.[14] The commonest symptoms of COVID-19 include headache, loss of smell, dizziness and loss of taste.[15] Emerging studies show that all nervous system components are affected, including central, peripheral, and muscular. In the present study, frequent CNS and PNS symptoms encountered in patients with mild COVID-19 infection were myalgia (67.4%), loss of appetite (34.8%), headache (17.4%), loss of smell (17.4%), and loss of taste (15.2%). Other non-specific symptoms include fever, cough, fatigue, and dyspnoea. In both the control and the study group, ART was lesser (faster) than VRT (Table 2 and 3). Two reasons could explain this. One is that males' temporal lobe is more well-developed than the occipital lobe and the second reason is the presence of multiple synapses in the visual (20-40ms) than in the auditory (8-10ms) pathway.[16] Normally males have a faster RT when compared to females due to stronger motor performance.[7] Slower RT in females might be due to estrogen and acetylcholine synthesis resulting in delayed nerve conduction. However, no such significant difference was present statistically in the present study, though mean VRT values are slightly more in males (Table 4).[17] Also, in the present study, the reaction time for red is a little faster than green in both the control and the study groups. This is in agreement with the previous studies conducted on VRT for different colours.[18] Sudden onset sensorineural hearing loss was also reported after COVID-19.[19] Reports as well had shown that mild acute COVID-19 patients who were never hospitalized had persistent neurological symptoms (long haulers), and no correlation was observed between disease onset and recovery time.[20, 21] In the present study, we also checked using AVRT whether the neurological issues were lasting after four and 8weeks of clinical recovery. It was observed that after four weeks of recovery from illness, reaction times were still prolonged when compared with the control (Table 2 and 3). However, the values in the study group were lesser than those during COVID-19 disease. This is an indication that the nerve function is improving. After eight weeks of COVID-19 recovery, the reaction time values in the study group were almost equal with the control group, showing the immense improvement in nerve function in two months. This was the first study to observe the neurological recovery in post-COVID-19 patients using the audiovisual reaction time.

Limitations of study

A larger sample size including not only mild, non hospitalized patients but also moderate and severely ill patients is needed to reach more accurate information. The study could have been extended for a few more months to obtain a complete recovery. Advanced neuroimaging techniques like MRI EMG-NCV were not done to support the findings. The reaction time varies with the phases of the menstrual cycle in females. However, this was not considered in the present study as only a limited number of patients volunteered for the study.

5. Conclusions

The present study results show that both ART and VRT for green and red colour were prolonged during COVID-19 disease. This specifies the involvement of the nervous system in COVID-19 infection irrespective of whether the patients came with neurological or respiratory symptoms. The values of ART and VRT for both the colours gradually decreased over 4 and 8 weeks of recovery, indicating improvement in nervous function. Even then, the ART and VRT values were slightly prolonged compared to the control. These preliminary results are shared to inform that CNS involvement occurs in COVID-19 infection but might gradually resolve in mild cases. Early recognition and prompt treatment of COVID-19 cases are essential to prevent long-term neurological consequences.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Original Article

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Physiology Section

Effect of Mint Flavoured Chewing Gum in Observing Changes in Cognitive Function while Assessing Test Performance- An Interventional Study

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ABSTRACT

Introduction: Cognition is the mental process of acquiring knowledge and understanding through aspects such as awareness, perception, reasoning, memory and judgement. Chewing movement of jaw stimulates memory parts of brain by increasing blood flow and glucose delivery. Taste and odour of mint is also known to stimulate memory areas of the brain. The synergistic effect of chewing and flavour is expected to have a greater effect on cognition than chewing alone.

Aim: To assess the effect of use of mint flavoured, flavourless and absence of chewing gum on an individual's cognitive function among the medical undergraduates.

Materials and Methods: This comparative, interventional study, was conducted in the Department of Physiology at Velammal Medical College and Hospital, Madurai, Tamil Nadu, India, August 2019 to September 2019. Study involved 75 (39 females, 36 males) MBBS first year students, aged 18-20 years. Only students with cognitive score between 28-30 based on Mini-Mental State Exam (MMSE) score were included in the study and were divided into 3 groups. Group A (n=25) who were given mint flavoured chewing gum, Group B (n=25) given flavourless chewing gum and Group C (n=25) the control group, not provided with chewing gum. Baseline memory, Heart Rate (HR), Reaction Time (RT) and Stress Levels (SL) were recorded. Groups were taken into separate rooms where they were allowed to study a particular topic i.e Parkinson's disease for 30 minutes. Then they were allowed to take tests on standard Parkinson's questionnaire for 20 minutes and assessed based on the test performance. Group A and Group B were provided with chewing gums both during studying the topic as well as taking tests. Post intervention test performance (short term memory), HR, RT and SL were again recorded. Test performance was also assessed after one month to assess the effect of chewing gum on long term memory. One-way Analysis of Variance (ANOVA) and paired t-test were used to compare all the post test parameters between the three groups.

Results: A statistically significant increase in short term memory (p-value=0.001) and HR (p-value=0.001) were observed after intervention. Similarly, short term memory level of the three groups subjects statistically differed (p-value=0.001). When considering the reaction time (p-value=0.068) and stress level (p-value=0.927), there was no significant difference among the three groups after the intervention. Assessment of the test scores alone after one month (long term memory) showed a significantly higher score (p-value <0.001) in Group A when compared with the other two groups.

Conclusion: Mint flavoured chewing gum improves cognition as evidenced by improvement in test scores, alertness and attention. The performance in the flavour less chewing gum group was lesser than mint flavoured group, but significantly better than control group.

Keywords: Heart rate, Memory, Menthol, Reaction time

INTRODUCTION

Chewing gum is generally preferred for maintaining alertness and preventing sleepiness while studying or driving. It creates a sense of euphoria and helps rejuvenate especially flavoured ones. It tends to affect a range of cognitive functions including aspects of memory, selective and sustained attention, psychomotor speed and accuracy [1]. The chewing movement of jaw stimulates nerves and parts of brain leading to increase in cerebral blood flow [2]. This increased blood flow to brain enhances glucose delivery to memory associated regions improving both episodic and working memory [3]. Chewing also facilitates release of insulin which influences memory via central mechanism [4]. The process of mastication increases the sympathetic nervous system activity and decreases parasympathetic activity [5]. Chewing gums activates brain centres such as prefrontal cortex, middle frontal gyrus (Brodmann's area 9 and 46) in dorsolateral prefrontal cortex, right prefrontal motor cortex, precuneus, thalamus, hypothalamus and inferior parietal lobe which has an enhancing effect on memory [6]. Though the effect of chewing gum on cognition and memory was evaluated in previous studies, the results are still debatable [7-9].

Alertness refers to the ability to develop and sustain a state of mental readiness. Attention requires efficient perception, learning, memory, and reasoning. The parameters for measuring alertness and attention were heart rate and reaction time. Reaction time is the minimum time taken to respond to a stimulus and it helps in assessing the integrity of the nervous system. The effect of chewing produced changes in reaction time and Event Related Potential waveforms thereby improving the cognitive processing [10]. The role of chewing gum in altering alertness and attention has been proved in many previous studies [11,12]. Chewing of flavoured gum consistently increases the beta rhythm of electroencephalogram, which is supposed to be the rhythm associated with arousal and alertness [13]. However, few studies have also demonstrated the same arousal effect with flavourless and odourless chewing gum [6,10].

Stress induces the release of free radicals in the body and is considered to be the cause for various health related problems like atherosclerosis, endothelial damage, hypertension, asthma, irritable bowel syndrome, cancer [14]. Stress can impair the memory function [15]. The results of the previous studies on the effect of

chewing gum on stress were controversial [16,17]. Few studies had reported a reduction in stress level under acute social stress, whereas no reduction in anxiety was found after chewing gum in other studies [16,17].

Smell is perceived in widespread areas of the limbic system including hippocampus, entorhinal cortex, orbitofrontal cortex and amygdala. Perception of taste occurs in the insular cortex. Integration of smell and taste sensation happens in the orbitofrontal cortex. All the above mentioned areas are associated with learning and memory [18]. It is expected that mint flavoured gums have an arousing effect in these areas both via taste and smell modalities. Though researchers were unable to point exactly why chewing gum boosts memory, attention and cognitive reasoning skills, the results of different studies clearly showed that it does. Chewing gum has similar effect to that of strenuous activity which boosts test performances.

Hence, it was hypothesised that mint flavoured chewing gum can improve memory by increasing blood flow and stimulating memory areas of the brain which could lead to improved test performance. The aim of the present study was to determine the effect of mint flavoured chewing gum on short and long term memory, alertness, attention and stress among the medical undergraduates.

MATERIALS AND METHODS

This comparative, interventional study, was conducted in the Department of Physiology at Velammal Medical College and Hospital, Madurai, Tamil Nadu, India, August 2019 to September 2019, after obtaining Institutional Ethical Clearance (IEC No: VMCIEC/24/2019).

Sample size calculation: Sample size was calculated with Cohen's D effect size fixed as 0.49 with 95% confidence level and 80% power. The minimum sample size thus calculated was 16.

Inclusion criteria: The study was initially explained in detail to all 150 first year MBBS students. Willing 126 first year MBBS students in the age group of 18-20 years, of both the genders, were enrolled for the study.

Exclusion criteria: The students were then screened and those with dental problems (undergoing orthodontic treatment, having fixed appliances), refractive errors, ophthalmic lesions, common cold, having difficulty in mastication (temporomandibular joint issues), under medication were excluded from the study. Smokers and those who have the habit of chewing gums regularly (more than 6/week) were also excluded.

Study Procedure

After the initial screening, baseline cognitive functions of 91 students were assessed using MMSE tool and only those with scores between 28-30 were included in the study [19]. Total 16 students were excluded, as the score was less than 28.

After obtaining informed voluntary consent from the remaining 75 students, the participants were randomly divided into three groups, three types of paper lots were created using alphabets A, B and C of 25 each. All the 75 students were instructed to select one paper lot and based on that, they were grouped accordingly.

- Group A (n=25) was the mint flavoured chewing gum group,
- Group B (n=25) was the flavourless chewing gum group
- Group C (n=25) was the control group which were not provided with chewing gum.

Wrigleys extra long lasting flavour (sugar free) peppermint gum (Illinois, U.S) was used in the mint flavoured group and Wrigleys gum base (synthetic rubber) was used in the flavourless chewing gum group. Group A and B were the interventional groups where exposure (chewing gum) was assigned.

Description of Intervention

The subjects were instructed to refrain from caffeinated drinks (and other stimulants) and exercise on the morning of the test. The test was conducted between 8 am-1 pm in the Department of Physiology. Demographic and anthropometric data including age, gender, weight and height was collected from all the 75 participants. Informed written consent was obtained from each participant. They were allowed to relax for 5 minutes after which visual reaction time and heart rate were recorded for all the students. They were then instructed to fill the stress questionnaire. As the topic chosen for the study was Parkinson's disease, a 10 minutes introductory lecture on this topic was delivered to all the 75 participants simultaneously. The students listened without taking notes. It was also informed to all the participants priorly that they will be placed randomly in either groups.

After that, they were divided into groups and sent to three separate rooms. Now group A was given one piece of mint flavoured chewing gum and group B was given one piece of flavourless chewing gum. They were told to chew constantly while reading. Group C was not given chewing gum. The participants were instructed to read the Parkinson's disease topic from Comprehensive Textbook of Medical Physiology by G.K Pal, for 30 minutes [20]. After a resting period of 10 minutes, they were allowed to take tests for 20 minutes on the same topic using a standard questionnaire simultaneously [21]. Group A were provided again with one piece of mint flavoured gum and group B with one more piece of flavourless chewing gum while doing the test. Immediately after the test, while group A and group B were still chewing, visual reaction time and heart rate was recorded and the post stress questionnaire was filled. After a period of one month all the groups were intimidated to take the same test with the same set-up and the test performance results were analysed. During this one month interval, the participants were restricted from chewing gums. After one month, they were allowed to take chewing gum only during the test performance.

Data Collection Method and Tools

- **Measurement of reaction time** [22]

Visual Reaction Time (VRT) was measured with the help of discriminatory and choice reaction time apparatus (Anand Agencies, Pune). The VRT for light stimuli with an accuracy of 0.001 second was measured in the sitting posture in a quiet room [22]. To record the baseline VRT for light stimulus (red), initially the subject was instructed about the complete procedure. The subject was asked to keep pressing the response button of the visual stimulus using the index finger of right hand. He should remove his finger immediately after he sees the stimulus. The value of VRT in milliseconds was displayed on the screen. Sufficient time was given for the participants to get acquainted with the procedure thoroughly. After the practice trial, once the patient felt comfortable, three readings were taken and the fastest response value was taken as the final reaction time.

- **Heart rate**

The Heart rate was measured with the help of masimo pulse oximeter to assess alertness [23].

- **Stress**

The Stress was assessed with the help of perceived stress scale questionnaire [15]. This scale includes 10 questions and the scores:

0-13 is considered as low stress,

14-26 as moderate stress

27-40 as high stress.

The participants read the Parkinson's disease topic from the book Comprehensive Textbook of Medical Physiology by G.K Pal [20].

- **Short and Long term memory**

Both short term and long term memory was assessed using Parkinson's disease questionnaire [21]. This included 20 questions on definition, causes, features and treatment of Parkinsons disease. The present study used a modified version of this questionnaire including the same 20 questions but for a score of 20 marks. The questions were of open-ended text type and each correctly answered question carried one mark.

STATISTICAL ANALYSIS

The data was entered into Microsoft excel and analysed using Statistical Package for Social Sciences (SPSS) version 20.0. Descriptive statistics like mean and standard deviation were used to represent continuous variables. One-way Analysis of Variance (ANOVA) was used to compare more than three groups on the basis of mean and standard deviation scores. Bonferroni multiple comparison test was used to compare the combinations of two groups in ANOVA. Paired sample t-test was used to compare the scores of before and after intervention. A 5% level of significance was considered statistically significant (p-value <0.05).

RESULTS

To ensure that all the selected participants (based on MMSE score) had the same level of heart rate, reaction time and stress before the beginning of the test, baseline equality verification was done. Statistical analysis was done to ensure that baseline parameter values were similar for all the groups before intervention. The p-value was non significant (p-value >0.05) among all the three group subjects, showing same level of heart rate, reaction time and stress [Table/Fig-1].

After the intervention, three groups' subjects' heart rates significantly differed (p-value <0.001). Especially, Bonferroni test revealed that the heart rates of control group subjects (A vs C has p-value=0.001 and B vs C has p-value=0.001) significantly differed compared to that of subjects of the other two groups. Similarly, short term memory level of the three groups subjects statistically differed (p-value=0.001). When considering the reaction time (p-value=0.068) and stress level (p-value=0.927), there was no significant difference among the three groups after the intervention [Table/Fig-2].

After the intervention, there was a significant improvement in the heart rates in group A (p-value <0.001) whereas there was no significant change in the heart rates in group B (p-value=0.665). In addition, reaction time of the subjects who use mint flavoured chewing gum had significantly reduced after the intervention (p-value <0.01). Similarly, the reaction time group A and B had significantly reduced after the intervention (p-value=0.006, p-value=0.001, respectively). However, stress level of the subjects was not significantly changed after the intervention in all three groups [Table/Fig-3].

Memory scores levels differed significantly across various groups after the intervention. Mint flavoured chewing gum group had high memory score compared to other groups [Table/Fig-4].

Overall, there was a significant change among the three group subjects in terms of heart rates, short term and long term memory levels after the intervention (p-value=0.001). Therefore, the intervention had the effect on the heart rates, short term and long term memory levels.

Group A and B had the similar level of effect on heart rate (p-value=0.371). However, group A had more effect on short term memory level compared to that of group B (p-value=0.029) after the intervention. When considered the within the group variations after the intervention, subjects who used group A had significant improvement in the heart rates and reduction in the reaction time. Therefore, group A was better than group B in terms of significant improvement in heart rate, short term and long term memory levels.

Parameter	Group A Mean±SD (n=25)	Group B Mean±SD (n=25)	Group C Mean±SD (n=25)	F-Statistic	p-value
Heart rate (beats/min)	83.00±10.09	86.72±15.63	78.80±10.20	2.614	0.080
Reaction time (milli seconds)	212.36±37.05	219.96±29.02	198.32±33.97	2.683	0.075
Stress level	19.12±9.08	19.64±6.50	19.52±7.11	0.032	0.969

[Table/Fig-1]: Comparison of heart rate, reaction time and stress level among the three groups before intervention.
One-way ANOVA *p-value <0.05 was considered statistically significant

Parameters	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD	F-Statistic	p-value	Bonferroni, p-value		
						A vs B	A vs C	B vs C
Heart rate (beats/min)	93.36±10.55	88.20±15.63	76.08±7.46	14.342	0.001**	0.371	0.001	0.001
Reaction time (milli seconds)	193.84±29.04	197.44±23.77	178.16±37.52	2.799	0.068	1.000	0.224	0.088
Stress level	18.12±9.31	18.64±6.72	18.96±6.70	0.076	0.927	1.000	1.000	1.000
Short term memory level	13.80±5.80	10.54±3.63	7.40±3.09	13.600	0.001**	0.029	0.001	0.038

[Table/Fig-2]: Comparison of heart rate, reaction time, stress level and short term memory level among the three groups after intervention.
**p-value <0.01 will be considered statistically highly significant; STML: Short term memory level; One-way ANOVA, Bonferroni multiple comparison test

Parameters	Group A Mean±SD		Group B Mean±SD		Group C Mean±SD	
	Preintervention	Postintervention	Preintervention	Postintervention	Preintervention	Postintervention
Heart rate (beats/min)	83.00±10.09	93.36±10.55	86.72±15.63	88.20±15.63	78.80±10.20	76.08±7.46
	p-value=0.001**		p-value=0.665		p-value=0.021*	
Reaction time (milli seconds)	212.36±37.05	193.84±29.04	219.96±29.02	197.44±23.77	198.32±33.97	178.16±37.52
	p-value=0.006**		p-value=0.001**		p-value=0.002**	
Stress level	19.12±9.08	18.12±9.31	19.64±6.49	18.64±6.72	19.52±7.11	18.96±6.70
	p-value=0.244		p-value=0.223		p-value=0.265	

[Table/Fig-3]: Comparison of heart rate, reaction time and stress levels before and after the intervention within the groups while assessing the short term memory.
HR: Heart rate; RT: Reaction time; SL: Stress level; Paired sample t test; *p-value <0.05 will be considered statistically significant; **p-value <0.01 will be considered statistically highly significant

Group	N	Mean score of the test questionnaire (20 max)	Std. Deviation	F-value	p-value
Group A	25	8.2400	4.69734	8.681	0.001**
Group B	25	6.5800	2.64449		
Group C	25	4.0800	2.97097		

[Table/Fig-4]: Long term memory score after one month between the 3 groups.
**p-value <0.01 will be considered statistically highly significant; Statistical test used: one- way ANOVA

DISCUSSION

The act of mastication itself increases the blood flow to fronto-temporal cortex, caudate nucleus, thalamus, rolandic areas, insular cortex, cingulated gyrus and cerebellum as observed with xenon-enhanced computed tomography [24]. The temporomandibular joint movements due to chewing not only increases blood flow but also glucose delivery to mainly bilateral temporal cortical areas [2]. Since, the temporal cortex is associated with memory regions of the brain including hippocampus, activation of these areas occur. In the present study, test scores had increased significantly after chewing gum for 20 minutes in mint flavoured group indicating improvement in short term memory. Short term memory lasts for seconds to hours through processing mainly in hippocampus [25]. Since taste and smell centres are situated in the memory regions of the brain, chewing along with odour and taste of mint could have strongly activated memory areas of the brain improving the test scores in this group compared with the other two. This also explains the better performance in the flavourless chewing gum group compared with the non chewing control group. The results of the current study coincide with the results of the previous studies, where the test performance improved significantly in the gum chewing group when compared to the group which mimicked chewing movements and the group which did not chew gum [9,26]. But the present study results are contradictory to the results of a study done in 2008, where the chewing gum did not improve the short term memory performance scores [1].

Alertness and attention in the present study was checked by changes in heart rate and visual reaction time. A significant increase in heart rate was observed in mint flavoured group alone. This differs from the results of a chewing gum study where increase in heart rate was not observed, though there was increase in cortisol level and work performance [27]. The results of the present study is in accordance with many previous studies which had observed increase in heart rate due to chewing [1,9,27]. This was observed mainly during chewing and immediately after chewing. This increased heart rate by pumping more blood could have activated the memory regions of the brain. It could also be due to chewing associated increase in sympathetic activity and suppression of parasympathetic activity [4]. The decrease in heart rate in control group of present study could be due to increase in parasympathetic activity due to relaxation without any intervention.

In the present study, duration of visual reaction time decreased significantly within groups both in mint flavoured and in flavourless chewing gum group with no significant change between groups. This shows that an individual reacts faster to a visual stimulus due to the effect of mint flavoured chewing gum. The processing speed in brain had increased and this could be due to increased sympathetic activity and activation of ascending reticular activating system. The results of the present study coincide with the results of a previous study which showed quickened reaction time [12]. This quickening explains the increased activity in motor regions for alerting and executive networks especially anterior cingulate cortex and left frontal gyrus. Surprisingly reaction time significantly decreased in the control group and this could be due to the familiarity with the procedure when they did for the second time.

Stress scores did not change with chewing gum. In the present study, no stressful task was given to perform and post stress scores were assessed immediately after chewing gum for 20 minutes using a questionnaire. Regular gum chewing for 5 minutes, twice daily for 14 days had also resulted in significant decrease in stress level [8].

When all the three groups were assessed again with the same set of questions after a month for long-term memory, test scores were still significantly higher in mint flavoured group when compared with the other two groups. This could be due to stimulation of memory areas of the brain, mainly hippocampus, by the odour and taste of mint in the chewing gum. Hippocampus is essential for consolidating short term memory into long term memory and it was found that hippocampus is activated by mint flavoured chewing gum. The rate of chewing was not controlled and it was left to the choice of the participants as evidence indicates that more vigorous chewing does not modify the chewing effects on memory [27].

To confirm the effect of odour and taste in stimulating cognitive areas of the brain, a study was conducted by Hasegawa Y et al., where 25 healthy participants were divided into three groups-No taste/no odour chewing gum group, sweet taste/no odour gum group and sweet taste/lemon odour gum group. Cerebral blood flow was recorded during chewing using transcranial Doppler ultrasound and near infrared spectrometer while at the same time, bilateral masseter muscle activity was also monitored. Results revealed higher blood flow with sweet taste/lemon odour gum group compared to the other groups. This supports the additive role of both taste and odour in activating cognitive and motivational areas of the brain while chewing, than smell or taste alone [28]. A direct correlation was observed between peppermint oil aroma and improved memory by long term potentiation mechanism [29].

As it was recorded in a previous study that the effects of chewing gum started after 5 minutes of chewing and lasted for only 20 minutes, the present study participants were instructed to chew the gum for 20 minutes while studying and again for 20 minutes while doing the test [26].

Limitation(s)

The sample size was small. The results cannot be generalised as this study involved local medical students. Cross over between groups was not done. Heart rate and reaction time were not measured during long term memory assessment. Only subjective stress levels were assessed. Future studies may focus on measuring stress level after providing an exposure to acute stressor. Functional Magnetic Resonance Imaging (MRI) could be done to assess the changes in memory areas of brain. Further studies are also needed to study the long duration of gum chewing on memory.

CONCLUSION(S)

The present study results showed that mint flavoured chewing gum improved alertness and attention as shown by increase in heart rate and decrease in reaction time. Mint flavoured chewing gum improved memory as shown by the increase in test performance scores immediately as well as after one month. As participants chewed gum during learning and again during the test performance, their recall was improved by the taste and odour of mint which stimulated the memory areas of the brain. But the present study failed to show any improvement in stress level. Flavourless chewing gum improved memory, attention, and alertness when compared to the control group, without having any significant effect on stress level. Chewing mint flavoured gum before exams could help the younger generation perform better, as the amount of information to be processed and reproduced for students, especially medicos, is very huge. Mint flavoured chewing gums are cost-effective, easily accessible and can be chewed before the tests to improve cognitive function.

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Utility of biochemical markers in predicting severe COVID-19: experience from a tertiary hospital in South India

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ABSTRACT

Background

Coronavirus Disease 2019 (COVID-19) patients can present with a wide array of symptoms. For laboratory investigation of these patients several biochemical tests are routinely requested. Here we wanted to evaluate the utility of procalcitonin (PCT), ferritin, D-dimer, interleukin 6 (IL-6) and total lactate dehydrogenase (LDH) activity in predicting severe COVID-19 infection.

Patients and methods

This study was undertaken at a tertiary care medical hospital in Tamil Nadu, India representing 183 COVID-19 RT-PCR positive patients, who were grouped based on their disease severity as mild (n=21), moderate (n=115) and severe (n=47) cohorts. All routine

clinical chemistry analysis was performed as part of routine baseline assessment. Biomarkers of inflammation and infection were tested via the measurement of IL-6, PCT, ferritin, and D-dimer. Serum IL-6 concentration was estimated by ELISA, while total LDH activity was analyzed by kinetic colorimetric assay. Serum ferritin, PCT and D-dimer were measured by fluorescent immunoassay by sandwich immuno-detection method.

Results

Biomarkers were significantly different among subgroups, and the highest concentrations were found in those with intensive care unit (ICU) admission. Serum PCT showed the best power to predict the need for ICU treatment followed by D-dimer, IL-6 and total LDH. Based on the AUC-ROC analysis, mortality was most effectively indicated by D-dimer followed by PCT, LDH, IL-6 and ferritin.

Conclusion

Our study highlights the utility of some routinely available biochemical tests in the management of severe COVID-19. The higher baseline values of these biomarkers hint towards the probability of severe infection and a larger risk of death.

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INTRODUCTION

Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) has gripped the world after being first reported in Wuhan, China in December 2019. An enveloped single stranded RNA virus belonging to the family Coronaviridae and subfamily of orthocoronavirinae was isolated as the cause of the pandemic [1,2]. Since millions of people across the globe have been prey to this infection and have succumbed due to it. A highly populous country like India with low sanitation levels has been an easy target. The Coronavirus Disease 2019 (COVID-19) patients can present with a wide array of symptoms, which include mild fever, cough, fatigue, upper respiratory symptoms and gastrointestinal symptoms. Anosmia and dysgeusia have been reported to be frequently found in these patients. Some cases can develop severe complications, such as Acute Respiratory Distress Syndrome (ARDS), respiratory and cardiac failure leading to multiorgan dysfunction and death [3]. Early therapeutic intervention and continuous monitoring during therapy play a critical role in reducing mortality.

Evidence accumulated in recent past has suggested the critical role of cytokines and chemokines released due to cellular destruction caused by rapid viral proliferation [4]. The molecular testing forms the basis for diagnosis, but the requirement of sophisticated instruments and unavailability of trained personnel for performing Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) has been challenging. Several biomarkers are being utilized to predict severity of the disease. Inflammatory markers like Procalcitonin (PCT), C-reactive Protein (CRP) and interleukin-6 (IL-6) are being reported to be associated with the severity of COVID-19 infection [5]. Liver enzymes and renal functions are also monitored in patients suffering from COVID-19 [6,7].

Several biochemical tests are being performed in COVID-19 subjects. Risk stratification of COVID-19 cases can be done using the array of biochemical tests available. Hence, it is desirable to find early and effective predictors of clinical outcomes in these patients. Patients with severe COVID-19 presented with an immunochemical profile like in cytokine storm. The intensified production of pro-inflammatory cytokines may be involved in pathophysiology causing severe pulmonary oedema, respiratory failure and damage to organs, such as liver heart

and kidney [8]. Increase in pro-inflammatory cytokines e.g., IL-6 and tumour necrosis factor-α (TNF-α), have been observed in patients with severe disease and found to be significantly associated with mortality [9]. PCT is a routinely used inflammatory marker in the daily routine. Any microbial infection can cause a significant raise in PCT, as endotoxins and pro-inflammatory cytokines induce its release from parenchymal tissues. Various studies have supported the theory that considerable increase in PCT levels from its baseline value denotes the beginning of critical phase of COVID-19 infection [10]. Formation and lysis of cross-linked fibrin gives rise to D-dimer. This reflects the activation of coagulation and fibrinolysis. Severity of COVID-19 symptoms are found to be associated with hemostatic abnormalities and elevated levels of plasma D-dimer values [11]. Ferritin, being an acute phase reactant, is linked to the underlying systemic vasculitis that cause lesions in major organ systems [12]. Lactate dehydrogenase enzyme (LDH) is present in numerous tissues throughout the body; thus, tissue damage easily leads to its serum elevation. LDH in COVID-19 cases is seen as a marker of lung injury in the initial stage of the disease [13].

The plethora of pathological processes in COVID-19 include hyperinflammation, cytokine storm, dysregulation of coagulation pathway, thereby producing a picture of systemic vasculitis leading to varied fatal complications. Our study was to assess the utility of widely used biochemical parameters in predicting the severity and mortality in COVID-19 infection. We aimed to define the relative cut-off values for various biomarkers to foretell disease morbidity in COVID-19 infected individuals.

MATERIALS AND METHODS

This clinical study was carried out by the Department of Biochemistry, in a tertiary care medical college hospital located in Madurai, India. Consecutive adult patients with positive RT-PCR results were enrolled at this hospital from August 2020 to October 2020. The study was approved by the Institutional ethics committee. This study is in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki. The patients were grouped according to their clinical symptoms into mild cases as group I, moderate cases were grouped as group II and severe cases were grouped as group III, based on National Clinical Management Protocol COVID-19, Revised version 3, dated June 13, 2020, by the Ministry of Health and Family Welfare, Government of India. According to the guideline, patients with uncomplicated upper respiratory tract infection, and mild symptoms, such as fever, cough, sore throat, nasal congestion, malaise and headache were categorized as mild cases, who could be managed at home. Pneumonia with no signs of severe disease with presence of clinical features of dyspnoea and or hypoxia, fever, cough, including SpO_2 <94% (range 90-94%) on room air, respiratory rate more or equal to 24 per minute were categorized as moderate cases. Severe cases were those patients who developed severe pneumonia or ARDS with severe hypoxia. Patients who presented with sepsis or acute life-threatening organ dysfunction caused by an unregulated host response to suspected or proven infection were considered as severe cases. Patients presenting with persisting hypotension despite volume correction or even after correction with vasopressors were also grouped as severe cases [14].

Exclusion criteria

Subjects showing negative RT-PCR results for COVID-19, or having a history of any hepatic and renal diseases prior to being infected with viral pneumonia were excluded. Pregnancy and

the presence of malignancy were exclusion criteria as well.

Inclusion criteria

All adults, who were tested for COVID-19 infection and had positive result by RT-PCR during the defined study period were included into the study.

Assignment of study group

Patients in the mild group I were treated with home quarantine. The moderate cases (group II) were admitted to the hospital and were treated in isolation wards. The severe cases assigned to group III required admission to intensive care unit (ICU). The patients were sub-grouped as survivors and non-survivors based on the mortality at the time of discharge from the health care facility for further analysis.

SARS-CoV-2 RT-PCR testing was done by a closed system, Truenat from Molbio diagnostics private limited, India on Truelab workstation. Qualitative detection of SARS-CoV-2 was done from upper respiratory specimens (nasopharyngeal swabs and oropharyngeal swabs) in our hospital. Results were calculated based on graphical analysis and cycle threshold (Ct) values. The Envelope (E) gene and Open Reading Frame-1 (ORF1) gene were targeted for detection of infection by commercially available kit, as per manufacturer's instruction [15].

Data collection

Clinical data included gender, age, time of admission and time of discharge. Routine biochemical and hematological tests were conducted to assess their baseline values. All routine clinical chemistry analysis like renal and liver functions, serum electrolytes, complete blood count were performed as part of the routine baseline assessment. The routine clinical chemistry tests were performed using Toshiba 120FR fully automated system for baseline assessment of the patients. Biomarkers of inflammation and infection were tested, which consisted of IL-6, PCT, ferritin, and D-dimer. Serum IL-6 was estimated using a commercially available human IL-6 ELISA kit (Biotech Diaclone, Besançon, France) with a sensitivity of 2 pg/mL as per the manufacturer's instructions [16]. Serum ferritin, PCT and D-dimer were measured by fluorescent immunoassay by sandwich immuno-detection method using i-Chroma analyzer [17]. Total LDH activity was analyzed by kinetic colorimetric assay.

Statistical analysis

Data was analyzed using IBM SPSS v.16.0 statistical software. The non-normal distribution was confirmed by subjecting data for Kolmogorov-Smirnov test. Continuous variables with non-parametric distribution were expressed as the median (25th percentile, 75th percentile). Mean values with standard deviation were used to express data that was continuous and equally distributed. The categorical variables were summarized as frequencies and percentages. The data were compared between the groups based on severity of COVID-19 infection by using ANOVA and K independent sample test for parametric and non-parametric distribution, respectively.

Students unpaired t-test and Mann-Whitney U test were used for two-group comparisons of continuous variables in different groups based on the mortality. Statistical significance was assumed if $p < 0.05$. Receiver Operating Characteristic (ROC) curve analysis was performed to determine the diagnostic utility of various biomarkers of COVID-19 for determining ICU admission and for predicting mortality. The measures of diagnostic accuracy including the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio and negative likelihood ratio were calculated using MedCalc's diagnostic test evaluation calculator [18].

RESULTS

A total of 183 patients were included into the analysis, 69% (n=126) were males and 31% (n=57) were females. The patients were divided based on the severity of their disease as group I (n=21), group II (n=115) and group III (n=47). The mean age of the study population was 57.89 ± 14.3 years. Amongst our study population, 19.6% (n=36) died of the disease. There was no casualty in group I. Group II with moderate cases had a mortality rate of 10%, i.e., eleven cases. 53% of all cases (25 cases) from group III constituting severe cases, died. All statistical analyses and conclusions drawn are based on baseline values of the parameters studied. Table 1 shows the distribution of age, gender and biochemical markers amongst the three groups. All parameters showed difference across the 3 groups. P values for baseline characteristics and biochemical markers between the three groups are depicted in Table 1. All biomarkers were distributed in a statistically significant ($p<0.05$) manner amongst the groups. There was a statistical

Table 1 Distribution of baseline characteristics and biochemical markers of COVID-19 infected patients based on the severity of the infection

	Biological reference interval	Group I n=21	Group II n=115	Group III n=47	p value
Age (years)		47 ± 15	57 ± 14	62 ± 12	**<0.001***
Males [N (%)]		10 (48%)	88 (77%)	28 (60%)	
Total Protein (g/dl)	6-7.8	6.8 ± 0.6	6.4 ± 0.7	6.1± 0.8	**0.003***
Albumin (g/dl)	3.5-5.5	4 ± 0.3	3.7 ±0.4	3.6 ± 0.6	**0.002***
Sodium (mEq/L)	136-145	137 ± 4	135 ± 4	133 ± 6	**0.009***
Potassium (mEq/L)	3.5-5.0	3.9 ± 0.4	4.1 ± 0.6	4.3 ± 0.8	0.203
Chloride (mEq/L)	98-106	84 ± 42	100 ± 10	100 ± 6	0.857
Aspartate Transaminase (U/L)	Less than 35	30 (24, 58)	40 (34, 60)	51(35,69)	0.175
Alanine Transaminase (U/L)	Less than 35	24 (21, 36)	33(23, 53)	33 (25,56)	0.717
Alkaline Phosphatase (U/L)	36-92	80(69, 95)	73(59,101)	83 (64,106)	0.357
Urea (mg/dl)	17-43	22 (17, 27)	30(23, 42)	44 (29, 68)	**<0.001***
Creatinine (mg/dl)	0.7-1.3	0.8 (0.6, 0.85)	0.8 (0.6, 1)	1.0 (0.8,1.4)	**0.007***

Procalcitonin (ng/ml)	<0.1	0.1 (0.1, 0.1)	0.1 (0.1, 0.2)	0.3 (0.1, 0.6)	**< 0.001***
D-dimer (ng/ml)	<500	212 (165, 251)	382 (203, 743)	818 (368, 4490)	**< 0.001***
Interleukin 6 (pg/ml)	5.3 - 7.5	6.9 (5.1, 10.6)	50.8 (11.4,172.7)	144 (63.5, 32605)	**< 0.001***
Ferritin (ng/ml)	M: 20-250 F: 10-120	43 (18, 130)	328 (136, 536)	442 (188, 686)	**< 0.001***
Total lactate dehydrogenase (U/L)	60-100	514 ± 170	837 ± 378	1055 ± 539	**0.001***

*Notes: Data are mean ± SD and median (25th Percentile, 75th Percentile). *p <0.05 is significant, M: Males, F: Females.*

significance in the age distribution across the 3 groups, with older individuals having a higher disease severity.

The AUC-ROC curves were used for comparing the potential of different biomarkers such as PCT, D-dimer, IL-6, Ferritin and LDH to predict severity and mortality due to COVID-19, respectively (Figures 1 and 2). Accordingly, serum PCT had the best power to predict ICU admission followed by D-dimer, IL-6 and LDH.

Figure 1 **Receiver operator characteristic curves comparing the potential of biochemical markers to predict severity of COVID-19**

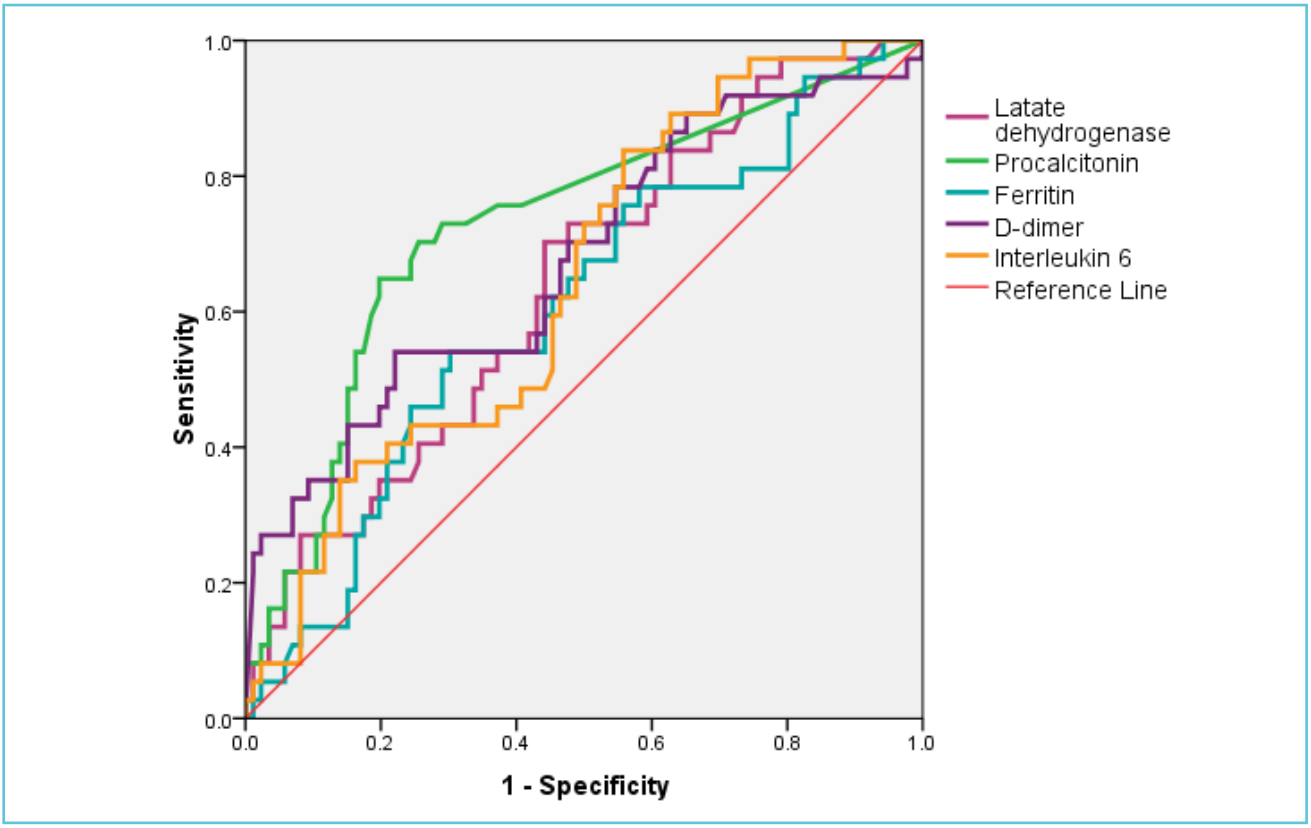
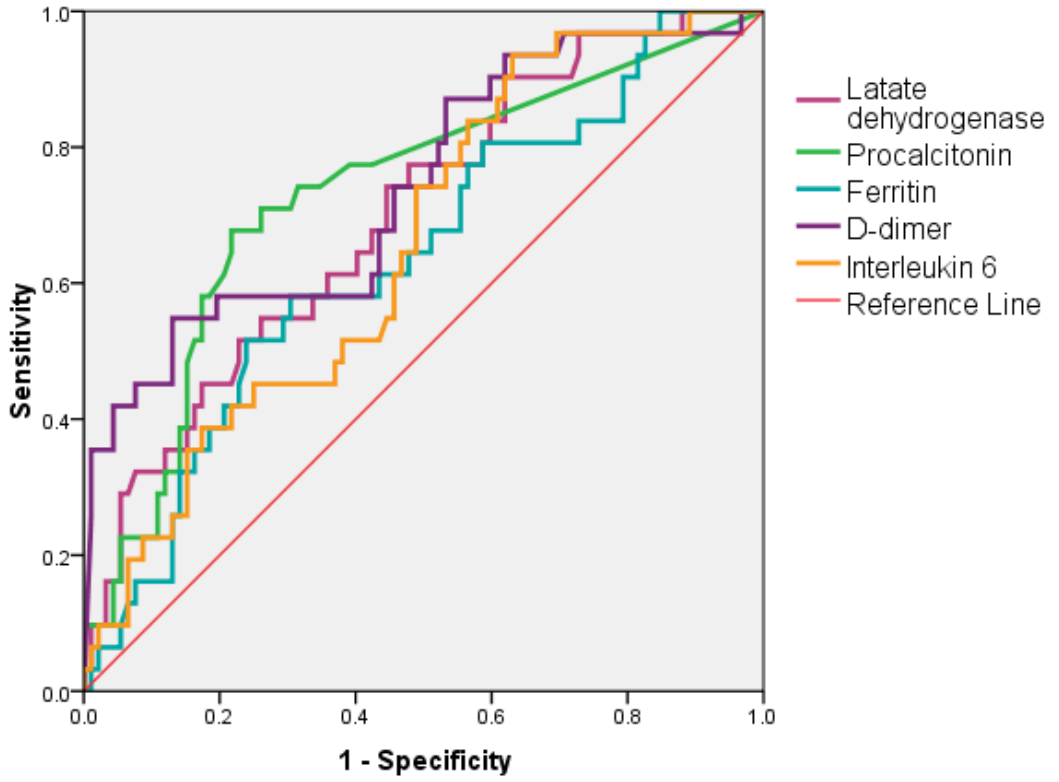


Figure 2 Receiver operator characteristic curves comparing the potential of biochemical markers to predict the mortality in cases infected with COVID-19



Based on ROC curves of biomarkers, comparison to predict mortality was done by analyzing the measures of diagnostic accuracy as displayed in Table 2. The ROC curve was used to obtain a specific cut-off for each biomarker. PCT had a sensitivity of 71% and a specificity of 70.7% at 0.15 ng/ml. Ferritin had a sensitivity of 58.1% and a specificity of 56.5% at 448 ng/ml. IL-6 showed a sensitivity of 74.2% and a specificity of 44.6% at 60 ng/ml, while D-dimer had a sensitivity of 58.1% and a specificity of 70.7% at 684 ng/ml. Finally, total LDH had a sensitivity of 77.4% and a specificity of 52.2% at 794 U/L (Figure 1).

PCT and D-dimer are seen to have better performance in comparison to IL-6, LDH and ferritin with respect to their AUC-ROC (Figure 1 and Table 2). D-dimer is seen to have best NPV followed by PCT to predict mortality. IL-6 was seen to have the highest PPV to predict mortality. The positive likelihood ratio for mortality prediction was seen to be best with IL-6 followed by PCT.

The distribution of biochemical markers among COVID-19 patients grouped based on their outcome are presented in Table 3. We found non-survivors to be significantly older than survivors.

DISCUSSION

This study is a retrospective study which was conducted to analyze the usefulness of some routinely available biochemical markers in the management of COVID-19 infection. Patients infected with SARS-CoV-2 infection tend to develop ARDS which requires early detection and monitoring from initial stages to prevent poor outcomes.

Table 2 Diagnostic performance of different biomarkers based on their cut-off value from the ROC curve analysis for the prediction of mortality in COVID-19

Biomarker (Cut-off)	AUC (95% CI)	p value	PPV (95% CI)	NPV (95% CI)	Positive Likelihood Ratio (95% CI)	Negative Likelihood Ratio (95% CI)
PCT (0.15 ng/ml)	0.731 (0.625, 0.838)	**<0.001***	71.43% (56.39-82.86)	70.25% (65.45-74.64)	3.89 (2.01-7.53)	0.66 (0.53-0.82)
Ferritin (448 ng/ml)	0.637 (0.525, 0.749)	**0.022***	55.56% (41.36 - 68.9)	62.61% (57.94-67.05)	1.75 (0.99 –3.09)	0.83 (0.69 -1.01)
IL-6 (60 pg/ml)	0.656 (0.551, 0.760)	**0.010***	80.56% (65.67-89.97)	58.22% (54.43-61.91)	4.23 (1.96-9.17)	0.73 (0.63-0.86)
LDH (794 U/L)	0.699 (0.594, 0.803)	**0.001***	72.22% (57.54-83.3)	56.07% (51.21-60.82)	2.49 (1.3-4.78)	0.75 (0.62-0.91)
D-dimer (684 ng/ml)	0.741 (0.636, 0.847)	**<0.001***	58.33% (44.7-71.24)	72.57% (67.55-77.07)	2.61 (1.48-4.62)	0.71 (0.56-0.90)

Abbreviations: AUC, area under the curve; ROC, receiver operator characteristic; positive predictive value (PPV), negative predictive value (NPV), PCT, Procalcitonin; LDH, Lactate dehydrogenase; IL-6, Interleukin 6.
**p <0.05 is significant.*

Table 3 Distribution of biochemical markers of COVID-19 infected patients grouped based on the outcome

	Survivors (N=147)	Non-Survivors (N=36)	t/z value	p value
Age (years)	56 ± 14	63 ± 10	-2.502	**0.013***
Total Protein (g/dl)	6.4 ± 0.7	6.2 ± 0.2	1.578	0.117
Albumin (g/dl)	3.7 ± 0.4	3.6 ± 0.4	1.337	0.183
Sodium (mEq/L)	135 ± 4	133 ± 7	2.270	**0.025***

Potassium (mEq/L)	4.1 ± 0.5	4.4 ± 0.8	-2.376	**0.019***
Aspartate Transaminase (U/L)	40 (31, 57)	54(40, 78)	-2.357	**0.018***
Alanine Transaminase (U/L)	31 (23, 49)	39(27, 64)	-1.565	0.118
Alkaline Phosphatase (U/L)	74(59, 102)	86(64, 101)	-0.986	0.324
Urea (mg/dl)	29(22, 41)	47(30, 71)	-4.552	**< 0.001***
Creatinine (mg/dl)	0.8 (0.6,1)	0.9(0.8, 1.3)	-2.184	**0.029***
Procalcitonin (ng/ml)	0.1(0.1, 0.2)	0.3(0.1, 0.7)	-4.486	**< 0.001***
D-dimer (ng/ml)	327(195,671)	1638(439, 9477)	-4.985	**< 0.001***
Interleukin 6 (pg/ml)	31.1(9.79, 164.4)	145(76.6, 312.3)	-4.371	**< 0.001***
Ferritin (ng/ml)	241(94,519)	619(313, 768)	-3.334	**0.001***
Lactate dehydrogenase (U/L)	820 ± 375	1133 ± 576	-3.714	**< 0.001***

*Note: Data are mean ± SD and median (25th Percentile, 75th Percentile). *p <0.05 is significant.*

Our study aimed at finding the utility of biomarkers for detecting severity of disease and predict disease outcome. We compared various biochemical tests among sub-groups based on disease severity. Five candidate biomarkers (Ferritin, PCT, IL-6, LDH and D-dimer) were chosen for comparison of their ability to predict severity and morality due to COVID-19 infection. We found that D-dimer and IL-6 had a vast difference between mild and severe cases. Similar finding was seen between survivors and non-survivors. PCT and D-dimer had a higher AUC-ROC curve for predicting severity and mortality as compared with other biomarkers.

Several studies have established that COVID-19 infected patients presented with pneumonia like symptoms [19,20,21]. The blood studies in COVID-19 infected cases at our tertiary care hospital revealed elevation of various inflammatory markers, such as IL-6, ferritin, D-dimer and PCT, which were comparable to previous reports [5,21,22]. ARDS associated with vast production of inflammatory cytokines, resulting in multi-organ dysfunction in viral infections resembles the features of secondary hemophagocytic lymphohistiocytosis (HLH) [23]. Such proinflammatory response due to exuberant elevation of cytokines has been previously documented in COVID-19 infections [24].

IL-6 is a pleiotropic cytokine, secreted by cells of innate and adaptive immune system as a response to microbial antigens. It causes enhanced activity of T and B cells, neutrophils and monocytes by triggering JAK2-STAT pathway. It induces the secretion of CRP, which helps in activation of classical complement pathway, thereby facilitating mediation of phagocytosis. IL-6 has been proposed to be a good marker of

prognosis in COVID-19 [5,25]. IL-6 contributes to the effective host defense against SARS-CoV-2 infection. However, extensive production of IL-6 can lead to cytokine storm which encompasses severe systemic inflammatory response [26]. IL-6 blockade therapy, using humanized anti-IL-6 receptor antibody, tocilizumab has been found to be beneficial in treating COVID-19 infections [25]. In our study, IL-6 levels were found to be elevated significantly in group III (severe COVID-19 infection). This was similar to the findings in a meta-analysis by Henry et al. [27] and Parsons et al. [28] suggesting the use of IL-6 as a biomarker for prognostic monitoring.

Bacterial infections stimulate amplified production of PCT from extrathyroidal tissue. In viral infections increased interferon-γ inhibits PCT production to remain it in normal limits in non-complicated cases of COVID-19 [28,29]. PCT is more likely to make a distinction between bacterial infection and other inflammatory processes than total leucocytes count or CRP levels [30]. We found PCT to be elevated in severe cases of COVID-19 infection as proposed by previous studies [4,30]. PCT is a crucial biomarker which if elevated at the time of hospitalization may be suggestive of severe COVID-19 infection.

Although lungs are the main target organ for COVID-19, kidneys and liver have been frequently affected due to the hyperimmune response caused by the virus [29]. Angiotensin converting enzyme-2 (ACE-2) receptors are known to ease binding of the virus and help in its entry into the cells [31]. ACE-2 receptors are present abundantly in small intestine, heart muscle, kidney, testis and thyroid [5]. The expression of ACE2 receptors on the renal tubules makes them a target organ for the virus [30]. Renal functions were seen to deteriorate with severe infection. The cholangiocytes have a higher expression of ACE2 receptors, thereby making them a suitable target for SARS-CoV-2 resulting in hepatic dysfunction. The mechanism proposed for transitory elevation in transaminases in COVID-19 infection is secondary liver damage due to hyperinflammatory response to infection. This can also be due to hepatotoxic drugs being used in the management of these patients [22]. Previous studies by Ferrari et al. [32] and Kumar et al. [6] claimed significant levels of elevation of transaminases in severe COVID-19 infections, whereas our findings did not show a statistical significance in the levels of transaminases amongst COVID-19 cases.

Serum ferritin, a marker of iron storage in the body, is seen to increase in cases of inflammation, hepatic disorder and malignancy [4]. It has been increased in patients with severe infection due to COVID-19 as a result of associated secondary HLH and cytokine storm [33]. Controlling of availability of iron to pathogens by ferritin plays a significant role in protecting the body against active infection [31]. Increase in ferritin levels is typically in the range of 500-3000 ng/mL. The increase in ferritin levels leads to activation of endothelial cells in the pulmonary vessels. This can cause imbalance in the normal hemostasis, regulation of fibrinolysis and maintenance of permeability of the vasculature. Such imbalance has a function in the development of COVID-19 vasculopathy resulted by inflammation [34]. The lower respiratory tract injury in COVID-19 patients explains elevated LDH levels. LDH being an indicator of lung injury, increases proportional to the severity of infection [26].

Hyper inflammation leading to elevated D-dimer and fibrinogen levels were seen to cause hypercoagulation and various complications such as Disseminated Intravascular Coagulopathy (DIC) [29]. D-dimer levels were seen to be higher in patients with severe infection as compared with milder infection of COVID-19. Such findings have been described earlier by Ponti et al., who suggested the activation of coagulation and secondary hyperfibrinolysis in mortality due to

COVID-19 infection [30]. D-dimer levels indicated thrombosis and elevated Fibrin Degradation Products (FDP) that occur due to thrombolysis [5]. Administration of anticoagulant therapy with low molecular weight heparin has been reported to be associated with a better prognosis due to decreased venous thromboembolism and DIC.

In our study, IL-6 levels were found to be elevated significantly in in non-survivors. Tjendra et al. studied various biomarkers to predict severity and outcomes in COVID-19, stated that patients with IL-6 >10 pg/ml had a concurrent elevation of various other biomarkers. Such candidates were more likely to develop sepsis and eventually die within 3 days of hospital admission [35].

Non-survivors showed higher values of PCT in our study which was similar to the findings of Gao et al. [20] and a meta-analysis by Malik et al. [22]. Haywood and colleagues studied hospitalization and mortality among COVID-19 patients and found that in-hospital mortality was related to abnormal level of biomarkers, such as lactate, creatinine, procalcitonin and platelet count [36]. Regarding laboratory changes in patients with fatal COVID-19, Henry et al. reported that elevation in the levels of certain biomarkers, such as IL-6, ferritin, PCT, LDH and D-dimer were often seen in cases with fatal COVID-19. PCT can serve as a marker of secondary bacterial infection, which could increase the probabilities of fatal outcome [27].

The non-survivors also exhibited increased serum creatinine and urea concentrations as compared with the survivors (Table 3). Non-survivors had significantly higher AST levels than other liver enzymes. AST with dominated increase was stated to reflect real liver injury [35]. Increased cytokine secretion, ACE2 receptor binding affinity of spike protein of the virus could be predominant cause of multiorgan injury in COVID-19 [37].

Elevation of serum ferritin could be either due to leakage from damaged cells or by active secretion from HepG2 cells and macrophages. Ferritin is seen to possess both immunosuppressive and pro-inflammatory effects [38]. The activation of monocyte-macrophage system causing inflammation is a primary cause of elevated serum ferritin. This supports the theory that diabetics are more prone to developing inflammatory storm which indirectly causes rapid worsening and a poor prognosis in COVID-19 patients [39]. Our patients exhibited high levels of ferritin in the severe and non-survivors of COVID-19 infection which was also observed in previous studies by Keddie et al. [29] and Aloisio et al. [40].

Li et al. evaluated the effect of serum LDH at admission and found it to be an independent risk factor for severity and mortality in COVID 19 cases. Under the influence of acute hypoxia or inflammation, due to lung infection, thrombogenesis and organ injury can occur, thereby making LDH an important marker in COVID-19 cases [41]. LDH is released from numerous tissues during death [29]. Bao et al. suggests LDH as a marker related to the risk of death in COVID-19 cases [42]. In our study, we found highest LDH levels in severe cases and among non-survivors.

Severe inflammation and hypoxia due to pneumonia cause activation of coagulation and fibrinolysis resulting in hypercoagulation state leading to DIC and multi-organ dysfunction. Zhang et al. have studied D-dimer in COVID-19 patients and concluded that D-dimer > 2µg/ml at baseline could predict in hospital mortality [11]. In addition, these patients were at a higher risk of developing pulmonary embolism. Malik et al. opined that elevated D-dimer was related to poor outcomes in COVID-19 patients [13]. Presence of prothrombotic milieu in non-survivors of COVID-19 infection could be the cause of elevated D-dimer levels. Patients with

severe infection and non-survivors exhibited higher levels of D-dimer. Thus, D-dimer is a reliable indicator of severity and can indicate outcome of the infection. This finding is supported by Ye et al. who suggested dynamic monitoring of D-dimer in hospitalized COVID-19 cases to monitor the risk of death [21].

Our observations reflect the efficacy of various biochemical markers. Biomarkers were significantly different amongst all groups and those with ICU admission had the highest concentrations. Serum PCT had the best power to predict ICU admissions followed by D-dimer, IL-6 and LDH (Figure 1). The areas under ROC curve was highest for D-dimer to predict the mortality followed by PCT, LDH, IL-6 and ferritin (Table 2). Finally, D-dimer is a better candidate amongst the chosen biomarkers based on its AUC-ROC curve for predicting mortality.

Our study being retrospective in nature is associated with few limitations. The lack of serial monitoring of various biomarkers is a drawback of our study. This was mainly due to the protocol followed at our institute that comprised of baseline laboratory assessment and continuous clinical monitoring. The inadequate knowledge about SARS-CoV-2 during the initial days were reasons behind such practice. Larger prospective studies with clinical correlation will help us to obtain valuable insights in the disease management and patient outcomes. One other limitation was that the study population included patients with comorbidities such as diabetes, hypertension, overweight, etc., which could also influence the severity and mortality of COVID-19. The sample population being heterogeneous in nature adds weightage to the study. We opine periodic monitoring of biomarkers among COVID-19 patients may aid the early detection of worsening of disease status. This can assist in timely escalation of the treatment protocol, which could be potentially lifesaving.

In conclusion, the higher baseline values of these biomarkers hints towards the probability of severe infection and increased mortality. Baseline biochemical markers help in segregation of high-risk cases and improve the management of patients resulting in an overall improvement. Stratification of cases helps in better management of hospital resources, manpower and aids early identification of requirement of ICU care. Our study highlights the utility of biochemical tests in management of COVID-19. The ease of testing makes them suitable for both triaging as well as monitoring of therapy.

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Evaluation Of Association Of Serum Leptin With Chronic Complications Of Diabetes And Glycemic Control

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ABSTRACT

Background: The chronic complications of type-2 diabetes mellitus have a major impact over the growing mortality and morbidity worldwide as well as in India. Leptin, an adipose-derived energy balance regulating hormone has been implicated in the development of chronic complications of type-2 diabetes mellitus. This study was done to clarify the association of leptins with the complications of diabetes

Materials and methods: An analytical cross-sectional study was conducted in 160 non-obese type-2 diabetic patients, of which 80 had one or more chronic complication of diabetes and 80 were without any complications. The fasting and postprandial sugar levels, serum leptin, HbA1c and renal parameters were measured.

Results: Leptin levels in diabetic patients with complication was found to be lower than that of patients without complications in both the gender ($p < 0.001$). There was no significant difference in the leptin levels among various complications ($p = 0.620$). We found an inverse correlation between leptin and fasting blood sugar levels ($r = -0.172$; $p = 0.030$), postprandial blood sugar levels ($r = -0.194$; $p = 0.014$) and HbA1c ($r = -0.271$; $p = 0.001$).

Conclusion: Our findings suggest that leptin might have a role in regulating the glycaemic status. Also, reduction in concentration of leptin is associated with the development of complications of diabetes.

Key Words: Chronic complications of diabetes mellitus, glycaemic status and leptin.

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both.[1] Despite being one of the most extensively explored diseases, acceleration in its prevalence never diminishes.

Type II diabetes mellitus is the commonest of all diabetes with a complex pathophysiology which result in hyperglycemia.[2] Patients with Type II diabetes mellitus can develop acute and chronic complication due to hyperglycaemia. The two types of chronic complication which can occur in type II diabetes mellitus are microvascular and macrovascular.

Leptin is an adipose-derived energy balance regulating hormone produced from OB (Lep) gene located on chromosome 7.[3] Several studies have been done to explore the non-hypothalamic action of leptin. As a result of these studies, a significant proportion of the riddle of peripheral action of leptin has been unravelled. One such is the role of leptin is glucose homeostasis.

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Defect in this adipo-insular axis is one of the causes for the development of type 2 diabetes mellitus. Several studies have shown in leptin sensitive persons, leptin controls the secretion of insulin to adapt glucose homeostasis to the body fat store and it also increases insulin sensitivity. Obese individuals have leptin resistance which results in loss of leptin control over insulin secretion and thus results in hyperinsulinemia. Chronic hyperinsulinemia leads to pancreatic β cell failure. Due to leptin resistance, insulin sensitivity is decreased, which also leads to superadded insulin resistance. Finally, the β cell failure and reduced insulin sensitivity lead to the development of type-2 diabetes mellitus.[4]

Leptin levels are influenced by several factors like gender, BMI, adiposity, insulin levels and drugs.[5] Due to the presence of several confounding factors, the results of the studies which associate leptin levels in diabetic patients are not unified. While some studies show that leptin level is elevated in diabetics, some other studies indicate that there is no change in leptin level and there are few more studies point out that leptin levels are reduced in diabetic patients.[6-9]

This abnormal variation in leptin concentration in diabetics has been linked with the development of diabetic complications. Leptin is said to have a pleiotropic effect on complications of diabetes. studies have shown that leptin increases the nitric oxide production, which protect against the development of diabetic nephropathy.[10] On the other hand, some studies disagree with this and state that leptin increases the activity of the sympathetic nervous system and oxidative stress thereby worsening the diabetic nephropathy.[11-13] In diabetic retinopathy, few studies have shown that elevated levels of leptin worsen diabetic retinopathy due to angiogenic effect.[14-17]

Leptin has also been implicated in the development of cardiovascular events. Pietersen et al study stated that leptin might have a role in the development of atherosclerosis.[18] But Smith et al in their study pointed out that leptin has a cardio protective effect by upregulating the RISK pathway (phosphatidylinositol 3-OH kinase (PI3K)-cellular Akt/protein kinase B (Akt) and p44/42 mitogen-activated protein kinase (MAPK) extracellular signal-regulated MAPK (Erk1/2) signalling cascades) which reduces the ischaemia-reperfusion injury.[19]

Though above studies have shown that leptin is elevated in obesity related type – 2 diabetes mellitus and its relation with that of its complications, but the results on leptin levels in non – obese diabetic patients still remain contradictory, as also its association with the development of diabetic complication and this becomes our research goal.

The present study is designed to evaluate the relationship between serum leptin with non-obese type-2 diabetes mellitus and its role in the development of chronic complications and also to find the association between leptin and glycemic status.

The specific goals were to estimate leptin concentration among non-obese diabetes patients, to evaluate the difference in leptin levels between diabetic patients with and without chronic complications and to check the relation between leptin and glycemic status.

Materials and methods:

It was a hospital based analytical cross sectional study conducted by the Department of Biochemistry ,approved by the Institutional Ethics Committee.

The study population consists of 160 non-obese diabetic patients in which 80 are free of diabetic complications and the rest of them had one or more chronic diabetic complication. Type -2 diabetes mellitus was diagnosed based on the history of treatment with oral hypoglycemic drugs with American diabetic association criteria for the diagnosis of diabetes. The study population was selected based on inclusion and exclusion criteria. Both male and female patients between the age groups of 35-55 years of age with and without chronic complications of type-2 diabetes mellitus like Diabetic retinopathy, nephropathy, foot ulcers, cardiovascular complications related to diabetes were included in the study.

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The exclusion criteria were the patients with any form of renal, cardiovascular, ophthalmic, peripheral vascular disease ad subjects with BMI (Body Mass Index) ≥ 23, patients on insulin therapy, known case of hypertension.

After obtaining a written informed consent physical examination was done, BMI was calculated by measuring the height in cm and weight in kg. Fundus examination was done by an ophthalmologist to rule out the presence of retinopathy, ECG was done to find out the cardiac complication and foot examination was done to confirm the presence of foot ulcers.

Serum, plasma and urine sample was collected to estimated plasma fasting blood sugar, plasma post prandial blood glucose, HbA1c, serum urea, serum creatinine and urinary microalbumin. Serum leptin was estimated by sandwich ELISA method using Leptin- ELISA kit from DIA Source.

Statistical Analysis:

All results are expressed as mean ± standard deviation (SD). The difference in various parameters between diabetics with and without complications was done using independent student t-test. Difference in serum leptin levels between diabetic complications was seen using analysis of variance (ANOVA). The correlation between leptin with glycemic status was done using Pearson's correlation. p - value less than 0.05 is considered statistically significant. All analysis was done using Statistical Package for the Social Sciences (SPSS) version 16 for windows.

Results

Our study population consisted of 160 patients with type – 2 diabetes mellitus. Out of 160 diabetic patients, 80 patients did not have any complications and rest of them had one or more chronic complications of diabetes mellitus. In the later subgroup, the main bulk of the population was contributed by diabetic retinopathy (10%), diabetic ulcer (13.12 %) and those patients with both diabetic retinopathy and nephropathy (12.5 %).

The difference in mean age of diabetics with and without complications was found to be insignificant (Table I). Our study population was recruited in such a way that the body mass index (BMI) of the entire population was in normal range according to Asian classification for BMI. There was no significant difference in mean body mass index between subgroups (Table I). The mean fasting blood sugar, post-prandial blood sugar, HbA1c and urinary microalbumin were high in diabetic patients with complications compared to patients without complications. There was no significant difference in urea and creatinine concentration between the groups. But mean urinary microalbumin concentration was high in diabetic with complication compared to patient without complication ($p < 0.001$) (Table I).

The mean concentration of leptin levels in males was 10.46 ng/ml and in females it was 14.02 ng/ml. The difference in the leptin concentration found between the genders was statistically significant (< 0.001).

There was significant difference in mean leptin concentration between diabetic patients with and without complications in both genders. Namely, in the male subgroup the mean leptin levels in patients without complications was 12.90 ng/ml which was found to be significantly higher than that of the mean leptin concentration in patients with complications which was 8.18 ng/ml. In female subgroup the mean leptin concentration in diabetic patients without complications was 17.55 ng/ml which was found to be higher than in female patients with complications which was 10.19 ng/ml (Table II; Figure I).

We had done Analysis of variance (ANOVA) to see whether there is any difference in mean serum leptin concentration between various diabetic complications and it was found that there was no significant difference seen within and between various subgroups of diabetic complications (Table III)

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Pearson correlation was done to verify the association of leptin with that of fasting blood sugar, postprandial blood sugar and HbA1c. Our study results revealed that fasting blood sugar, post prandial blood sugar and HbA1c, correlated inversely with leptin concentration (Table IV).

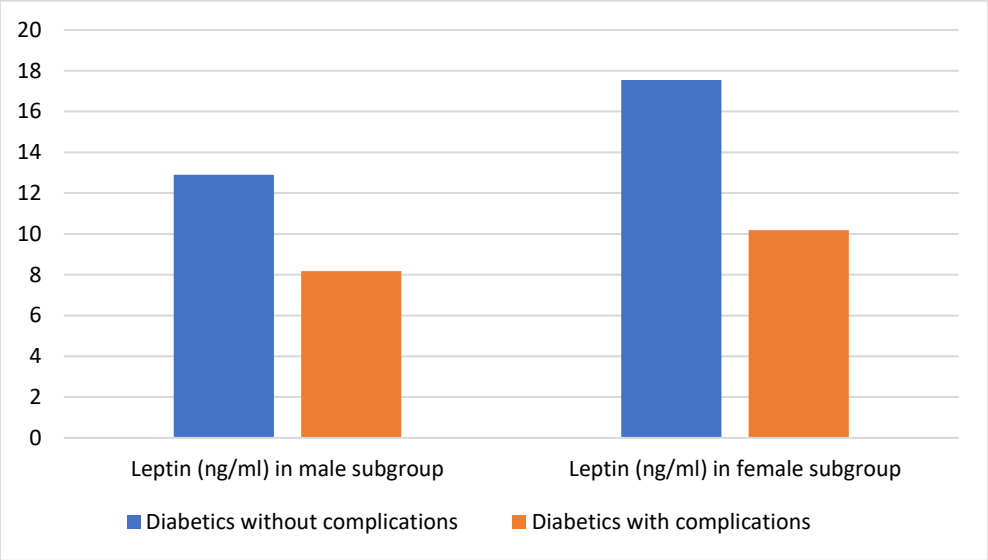

Figure I Gender specific difference in mean leptin variation among diabetic patients with and without complication

Table I: Demographic characteristics and laboratory findings of study population

Variables	Diabetics without complications n = 80	Diabetics with complications n = 80	p value
Age in years	45.75 ± 5.73	46.80 ± 5.60	0.243
BMI (kg/m^2)	21.42 ± 1.00	21.09 ± 1.36	0.085
FBS (mg/dl)	149.35 ± 61.94	176.26 ± 73.07	0.013*
PPBS (mg/dl)	237.21 ± 83.77	281.98 ± 106.95	0.004*
HbA1c (%)	7.67 ± 0.67	8.18 ± 0.95	< 0.001*
Serum urea (mg/dl)	25.48 ± 6.74	25.15 ± 6.20	0.741
Serum creatinine (mg/dl)	0.73 ± 0.15	0.77 ± 0.14	0.092
Urinary microalbumin (mg/L)	18.45 ± 4.92	47.83 ± 45.41	< 0.001*

* $p < 0.05$ is considered as statistically significant.

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Table II: Gender specific difference in leptin levels between diabetics with and without complications

Parameter	Diabetics without complications (Mean ± SD)	Diabetics with complications (Mean ± SD)	p value
Leptin (ng/ml) (Male Subgroup)	12.90 ± 1.89	8.12 ± 1.84	< 0.001*
Leptin (ng/ml) (Female Subgroup)	17.55 ± 1.79	10.19 ± 2.76	< 0.001*

** p < 0.05 is considered as statistically significant.*

Table III: Alteration in Leptin concentration in various diabetic complications

Subgroups	N	Serum Leptin Mean ± SD	p value
Diabetic retinopathy	16	8.70 ± 2.09	0.675
Diabetic nephropathy	11	8.04 ± 1.46	
Diabetic ulcer	21	9.39 ± 2.80	
Cardiovascular complications	6	8.92 ± 0.82	
Diabetic retinopathy and nephropathy	20	9.45 ± 3.29	
Cardiovascular complications and nephropathy	5	10.18 ± 1.50	
Diabetic ulcer and diabetic nephropathy	1	8.50	

* p < 0.05 is considered as statistically significant.

Table IV: Association between leptin levels with glycemic status.

Parameter	Leptin	
	Pearson Correlation r value	**p value**
Fasting blood sugar (mg/dl)	-0.172	0.030*
Post prandial blood sugar (mg/dl)	-0.194	0.014*
HbA1c (%)	-0.271	0.001*

* p < 0.05 is considered as statistically significant.

DISCUSSION:

The association between serum leptin levels and obese diabetic patients with diabetic complications has been studied extensively, but the studies which explains the relationship between leptin with that of non-obese diabetic patients and its complications are meagre and their results aren't unified. So, to clarify whether leptin alteration in diabetics is independent of BMI and also to explore its association with its complications we had conducted this study.

In this study, serum leptin concentration in the female sub group was greater than that of the leptin concentration in male study group population. The possible reason behind this variation in leptin concentration could be explained by differences in the sex hormone levels between male and female. Studies have shown that testosterone and oestrogen can alter the concentration of leptin. [20,21,22]

To rule out the gender influence over the result, we had divided the study population into two groups based on gender. The difference in leptin levels between diabetic patients with and without complications was done in each group separately. In both male and female subgroup, the leptin concentration in patients without complications is higher than that of leptin level in patients with diabetes complication. There was no difference in the leptin levels among the

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various complication of diabetes (Table III). So, our study postulate that leptin levels will be elevated in early stages and reduced in late stages of diabetes mellitus. This results of ours may be due to reduction in the β cell mass with severe insulin resistance in late stages of diabetes mellitus which result in reduced stimulation of insulin over leptin production.

Several studies have stated that leptin is reduced in late stages namely in patients with diabetic complications. And some of them had pointed out leptin might have a preventive role over the development of diabetic nephropathy, atherosclerosis, retinopathy. [19,23,26] In our study we found that leptin concentration is inversely correlating with glycemic status (FBS, PPBS and HbA1c). This finding in our study could be explained by the role of leptin over glucose homeostasis. Leptin increases insulin sensitivity, thereby reducing blood glucose levels. Leptin also reduces blood sugar levels by reducing hepatic glucose production. Even though leptin improves glycemic status, the magnitude of the increased glucose uptake elicited by leptin is generally much lower than that achieved by insulin.[27] This may explain the weak but significant association between leptin and glycemic status, which is seen in our study.

CONCLUSIONS:

From this study, we conclude that leptin might have a role in the control of glycemic status among diabetics and the development of diabetic complications is associated with reduction in leptin levels.

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Evaluation of Haematological Findings in Tuberculosis Patient of Madurai- An Cross Sectional Study

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Abstract

Background:
Hematological abnormalities are a common in pulmonary tuberculosis (PTB) patients, one of the major public health problems. However, the paucity of information about the hematological profile of PTB still exists. Many inflammatory cells, cytokines, acute phase reactants as well as platelets are recruited in the battle against the invading mycobacterium. As a result, alterations in the hematologic profile of infected patients are anticipated. Every year still nearly 2 million people are diagnosed with TB. Pulmonary TB constitutes nearly 70% of all the cause of TB in India. Pulmonary TB - with raised ESR, mild normocytic normochromic anemia and also an increased platelet count. ESR is a non specific marker of inflammation.

Objectives:
The main objective of this study is to investigate the various haematological characters of patients with active tuberculosis and extrapulmonary tuberculosis.

Materials & Method:
A total of 120 patients diagnosed as various forms of TB and treated in this institute from April 2020 till September 2021 were retrospectively studied and assessed.

Results:
Thrombocytosis was observed in 41.4% of patients, all of which were pulmonary sputum positive cases suggesting Thrombocytosis as an active inflammatory marker in active pulmonary Tuberculosis. Incidentally Thrombocytopenia was found in 4.84% of patients, all of whom were extra-pulmonary cases.

Conclusion:
The high incidence of Thrombocytosis in active Pulmonary sputum positive cases only, suggesting the presence of Thrombocytosis in smear negative Tuberculosis in patients who show radiological changes consistent with Pulmonary TB

Keywords: ESR, Thrombocystosis, Pulmonary And Extrapulmonary Tuberculosis

Introduction

Tuberculosis is a major public health problem in many countries globally. In India every year 14 to 16 lakh new patients are diagnosed and treated [(1)].

Diagnosis of the disease by sputum examination for AFB by ZN staining or Auromine-O staining under Flourescent microscopy or Myco bacterial Nucleic acid amplification tests by (CBNAAT) or Real time PCR (Trunat) still remain the basis for diagnosis of

active disease, aided by Culture by MGIT liquid medium or the conventional LJ medium as the gold standard of diagnosis.

But a lot of changes happen in the haematopoietic system in a person infected with Mycobacterium tuberculosis [(2)]. Though many studies have been conducted in this regard, still a complete haematological picture which can correlate with an active disease has not been revealed. Problems in diagnosis happen in some Pulmonary disease patients who can't bring out sputum for some reason or other, and in Extra-pulmonary disease where in some instances we can't get a tissue diagnosis and in Disseminated disease.

Though raised ESR was traditionally considered as a supporting parameter for active TB, the limitations of ESR in diagnosis of TB is well known, because ESR is a nonspecific inflammatory marker which is increased in a multitude of conditions, and has only prognostic value. Also many extensively involved case of TB can present with a normal or low ESR

Leucocytosis with either Polymorph increase or Lymphocyte increase along with Thrombocytosis have been reported along with Normochromic Normocytic anaemia. We have found in our observation over a period of time majority of active TB cases are having Thrombocytosis. Very little studies have been done with relation to C Reactive protein [(3)] [(4)].

Ours is yet another study in an attempt to correlate active Tuberculosis with the haematological characteristics changes with reference to CRP, Thrombocytosis etc and their relation vis-a-vis other haematological changes which can point towards a diagnosis of TB when a Bacteriological confirmation is elusive, for some reason or other.

Objective:

The main objective of this study is to investigate the various haematological characters of patients with active tuberculosis and extrapulmonary tuberculosis.

Materials And Method :

A total of 120 patients diagnosed as various forms of TB and treated in this institute from April 2020 till September 2021 were retrospectively studied and assessed.

Inclusion criteria:

Individuals > 12 years of age belonging to both sexes irrespective of presence of Diabetes Mellitus or any other Co morbid condition

Results:

A total of 120 patients were included in our study. Majority (70.7%) were male participants. Smoking history was present in around 80.3% of the male participants. Among the remaining female patients around 78.5% had history of passive smoking. In our study around 82.9% were pulmonary tuberculosis and 17.1% were extra-pulmonary tuberculosis patients.

Leukocytosis was observed in 29.3% of patients. Rest all had a normal value. Among the abnormal values around 91.7% were pulmonary tuberculosis rest 8.3% were extra pulmonary tuberculosis.

Lymphocytosis were found in 9.77 % of the patient and lymphocytopenia were observed in 56.09% rest had normal lymphocyte value. Similar pattern were found in Eosinophils and Basophils. The average value of all the blood parameters are enlisted in table 1

The platelet values are depicted in figure1.Thrombocytosis was found among 41.4% of the patients all those patients were pulmonary tuberculosis. Thrombocytopenia was found among 4.87% patients all were observed to be extra-pulmonary cases. The changes in the platelet counts were statistically significant among both groups of tuberculosis (p value- 0.001)

Among the participants only 46.34% had normal haemoglobin value rest all were anaemic. Table 2 explains the crosstab between the haemoglobin levels and the type of tuberculosis. There is a statistically significant change among both the groups (p value- 0.022)

In extra-pulmonary tuberculosis patients 14.28% was anaemic and 61.76% of pulmonary tuberculosis was anaemic.(Table 2)

Discussion:

In our study Leukocytosis was observed in 29.3% of patients. Rest all had a normal value. Among the abnormal values around 91.7% were pulmonary tuberculosis rest 8.3% were extra pulmonary tuberculosis. similarly in a study by rohini et al [(5)] WBC count in PTB subjects was increased ($p < 0.05$ for WBCs) and all were statistically significant. This

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study demonstrated that WBC count was increased when compared with healthy controls. In a study by yaranal et al [6] Leucocytosis as a response to infection was observed in 26 patients and three patients had leucopenia. while in contrast a study by shafee et al [7] Total leukocyte count was also lower than normal values in 8% and 6% of male and female respectively.

In our study Lymphocytosis were found in 9.77 % of the patient andlymphocytopenia were observed in 56.09% rest had normal lymphocyte value .while shafee et al [7] noticed Lymphocytopenia in 59% and 43% patients in male and female respectively

In our study Thrombocytopenia was found among 4.87% patients all were observed to be extra-pulmonary cases. The changes in the platelet counts were statistically significant among both groups of tuberculosis (p value- 0.001).while by yaranal et al [6] in his study observed Thrombocytosis in 24 patients while thrombocytopenia was observed in 9 patients.

In our study just more than 50% of patients were anaemic(53.66%). Of these 61.76% of Pulmonary cases and 14.28% of extra pulmonary cases were anaemic (Hb <11grams), suggesting a significant number of Pulmonary cases are prone to develop anaemia and had Normocytic Normochromic anaemia which is concurrent with the findings of other studies [8]. The high presence of anaemia in Pulmonary Tb cases could be attributed to the increased Bacterial load in Pulmonary cases, whereas extrapulmonary cases are paucibacillary. The extra Pulmonary cases we encountered were either Pleural effusion or Cervical lymphadenitis with a few cases of TB spine,some of which were detected by Gene Xpert(CBNAAT/Trunat)). Some cases of pleural effusion were treated as Tuberculosis despite CBNAAT negative, based on clinical judgement , correlating with other parameters, as we are aware that getting a positive Gene-Xpert in Pleural effusion is only 30% probabability, because pleural effusion in TB is mainly due to an allergic inflammatory process in Pleura secondary to a sub pleural focus of Koch infection.

We could not get to correlate the inflammatory markers like CRP as it had been done only In about 10% of cases, which is statistically inadequate data to correlate.

Conclusion

Our study-a retrospective analysis of Pulmonary and Extra-Pulmonary cases suggests the significant changes in Haematological indices especially in active Pulmonary cases , vis-a vis the extrapulmonary cases of TB suggesting the possible influence of High Bacterial load on the haematological characteristics of the patient.

One signifcant finding was the high incidence of Thrombocytosis in active Pulmonary sputum positive cases only, suggesting the presence of Thrombocytosis in smear negative Tuberculosis in patients who show radiological changes consistent with Pulmonary TB, could be an added tool to search for a bacteriological confirmation with Liquid culture (MGIT, BACTEC) or LJ medium culture, as we know Culture is the Gold standard for diagnosis of Pulmonary and Extra-Pulmonary TB.

In that aspect Thrombocytosis can be viewed as a haematological marker more than ESR because ESR is elevated in a multitude of conditions like Pneumonia, Bronchiectasis and a host of other lung diseases, other than Tuberculosis, and also in many systemic diseases and has only a prognostic value.

The limitation in our study is that the follow-up haematological values for the patients investigated, for none of them is available. If they had been done the value of Thrombocytosis as a Haematological marker in prognosis of the patients can be evaluated.

Further studies on the prognostic value of Thrombophilia in Pulmonary cases with progress under the influence of anti TB treatment, will throw more light on the importance of this haematological parameter.

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Table 1: Mean value of haematological parameters (n-120)

Haematological parameters	Mean ± SD
Haemoglobin	11.42 ± 2.02
Platelets	378780.49 ± 166524.85
Leukocytes	14646.34 ± 29058.82
Neutrophils	71.64 ± 11.07
Eosinophils	1.56 ± 2.122
Basophils	0.48 ± 0.436
Lymphocytes	17.51 ± 8.91

Table 2: Association between haemoglobin value and type of TB (n-120)

Haemoglobin	Type of TB		Value	P value
	Extra pulmonary	Pulmonary		
Normal	31.6%	68.4%	5.262	0.022
Anaemia	4.5%	95.5%		

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Clinico-radiological profile of bronchiectasis patients an observational study

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Abstract
Introduction: Bronchiectasis is a progressive, obstructive lung disease that results from the presence of chronic inflammatory secretions and microbes leading to the permanent dilation and distortion of airway walls. Continuous cough followed by shortness of breath will cause reduced quality of life among the patients. Despite so many techniques, etiology remains undetermined. Diagnosis of bronchiectasis relies on clinical suspicion. Hence this study is for performed to demonstrate the clinical and radiological profile of bronchiectasis patients.
Aim: To study the clinical and radiological profile of bronchiectasis and to assess the microbiological profile of bronchiectasis patients
Materials and methods: This was a hospital- based observational study conducted in the Department of Pulmonary Medicine in Gauhati Medical College and hospital, Guwahati. The present study was conducted from 1st August 2018 to 30th July 2019. All patients more than 14 years of age, with radiologically diagnosed bronchiectasis and willing to participate in the study were included. Demographic details of the patients were collected using a semi- structured questionnaire. Analytical tests were performed using Pearson correlation and Chi-square test.
Results: A total number of 127 bronchiectasis patients who meet the inclusion criteria were included in the study. The mean age of the patients was 49.47± 9.44 years. Common Etiology was idiopathic 54(42.51%) followed by history of antitubercular treatment 45 (35.43%). The most common radiological type of bronchiectasis was cylindrical 79 (62.20%) followed by cystic 36 (28.34%). Pseudomonas was the most common organism seen in 27 (21.25%) of the sputum cultures of bronchiectasis patients. Pseudomonas had a clinically and statistically significant association with cystic type of bronchiectasis with a p value of 0.0004.
Conclusion: In this study Idiopathic etiology is the common cause of Bronchiectasis. Obstructive pattern of spirometry is found in majority. Psedudomonas is the common pathogen.

Keywords: Etiology, Sputum culture, Spirometry

Introduction:

Bronchiectasis is an obstructive lung disease that results from the presence of chronic inflammatory secretions and microbes leading to the permanent dilation and distortion of airway walls (1) This may lead to recurrent lower respiratory tract infections, worsening pulmonary functions, respiratory failure and pulmonary hypertension, resulting in deterioration in quality of life, with increased morbidity and premature mortality. The word bronchiectasis is derived from Greek words, *bronchion* meaning windpipe and *ektasis* is stretching out. The etiology of bronchiectasis varies in different populations. Immune deficiency syndromes, metabolic and ultra-structural defects are the predominant etiologic factors in developed countries, while bacterial and viral infections continue to be major causes of the disease in developing countries (2) On the other hand, despite using advanced immunological and genetic diagnostic techniques, etiology in 40% of cases remains undetermined (3)

The diagnosis of bronchiectasis initially relies on clinical suspicion. More than 90% of patients had persistent cough and almost three-quarters describe daily expectoration of yellowish green purulent sputum. (1) The diagnosis of bronchiectasis is confirmed radiologically using high resolution computed tomography (HRCT) of the chest. It has become increasingly recognised that treatments for bronchiectasis cannot be extrapolated from other chronic respiratory diseases and more studies are needed to better understand disease pathogenesis and to establish an optimum approach to the management of this debilitating disease. There are very few studies done on bronchiectasis in India and as per my knowledge no study is reported from Guwahati, a north eastern city of the country. The present study was therefore planned to analyze the clinical ,radiological and microbiological spectrum of bronchiectasis in Guwahati, a north eastern city of the country. Our aim of the study was to find out common presenting symptoms, clinical signs, Chest X ray findings, HRCT Thorax patterns, Spirometrypatterns, microbiological profile and common etiologies of bronchiectasis in North Eastern region of India. This was a hospital based study done in Guwahati medical College & Hospital, a tertiary care hospital where patients come from different parts of north eastern region of India.

Materials and Methods:
Study subjects and study design

This study was a hospital based prospective observational study done in the department of pulmonary medicine, Internal Medicine and allied specialities in Gauhati medical College & Hospital, a north East Indian hospital. . Through simple random sampling

around 127 cases who attended the OPD from August 2018 to July 2019 were taken up for study. Written informed consent from the patients was taken. Ethical clearance (190/2007/pt-1/1EC/41) was obtained from Ethical committee of the institution prior to the onset of study.

Inclusion criteria: All patients more than 14 years of age with tram track appearance and with increased bronchovascular markings in chest X- ray were included . Patients with co-morbidities like carcinoma and pregnant patients were excluded from the study

Data collection

A preformed questionnaire was used to collect information regarding the demographic data, childhood history, symptomatology, and significant past and personal history. Chest radiograph, and high-resolution computed tomography (HRCT) chest was done to assess the radiological involvement. Sputum acid fast bacilli (AFB), bacterial culture and sensitivity was done to assess the microbiological colonisation. Bronchoscopy was done in selected patients and bronchoalveolar lavage was collected and subjected to AFB, bacterial culture and sensitivity were done as and when indicated. Spirometry with bronchodilator reversibility was done for airway assessment

Radiological diagnosis

Chest radiograph (postero-anterior view) was done in all patients, preferably in the standing position. The patients were advised to hold their breath for a few seconds. HRCT (FOV 35 cm , matrix size 768 * 768)

Spirometry

In spirometry flow-volume loop, FEV1 and FVC were recorded.The pattern of spirometry was classified into normal,obstructive,restrictive, and mixed types. Normal spirometry was defined as when the expiratory flow-volume loop had a triangular shape with its top at the left. The inspiratory part of the loop is shaped like a semi circle. The values of the parameters were higher than 80% of the predicted values, while the tiffeneau index (FEV1/FVC*100) was higher than 70. In Obstructive Lung Disease the tiffeneau index is below 70 and an indented or concave expiratory part of the flow-volume loop is found. When obstructive lung disease was present, often a post-medication test was performed after administration of a bronchodilator.

Statistical analysis

Statistical analysis was done using the Microsoft Excel and SPSS software. The chi-square($\chi 2$) test of independence was used to test for a statistically significant relationship between two categorical variables. P value ≤ 0.05, it was considered statistically significant. Pearson correlation was used to assess the strength of correlation between variables. The data were analyzed in tabular form, bar diagrams and pie diagrams as and where indicated.

Results:

This study had 127 patients with bronchiectasis. Among them 48.81% were seen in the age group of 46 to 55 years followed by 19.68% cases in the age group of 36 to 45 years. The highest age of the patient in the study was 69 years. The mean age of the patients was 49.47 ± 9.44 years. Overall, 56.69% cases of the study population were females and 43.31% cases were males.The female is to male ratio was 1.30:1.0. The most common presenting complaint was cough with expectoration (50.39%). Fever was the presenting complaint in 6.29% of cases whereas chest pain in 1.57%. In this study 62.99% of the cases were non-smokers. Overall, 42.51% cases did not have any significant respiratory illness in the past (table 1).

The obstructive pattern was most commonly found in spirometery (43.3%) (table 2).

Interpert table 3 to find the mostcommon pattern in Spirometry and HRCT.

Sputum microscopy for AFB was positive in 3.15% of cases. The most common microorganism isolated in sputum culture was Pseudomonas aeruginosa in 21.25%. Out of 27 patients with pseudomonas infection majority had cystic bronchiectasis. The association between the spirometric pattern and involvement of microorganism was significant for pseudomonas and hemophilus influenza (table 4)

No significant association was found among the spirometry pattern and the gender.

Discussion:

This study was carried out with the aim of assessing clinical, radiological and microbiological profile in bronchiectasis patients. Most significant observations and conclusions hence derived upon from this present study were in conformity with available literature on the subject.

Table 1- comparision of our study findings with other study findings.

characterstics	Our study	Other stdies
Mean age	49.47 ± 9.44 years	58 years- Angrill JC et 2002 (4) 57 ± 14years- king PT et al 2006 (5) 56 years- Habesoglu MA et al 2016 (6)
Smokers	19.68%	62.92% - Devi L et al 2018 (7)
Idiopathic etiology	42.51%	26.06%- Shoemark A et al 2007(8) 66%- Qi.Q,Wang et al 2015 (9)
Bilateral lower lobe involvemet in chest radiography	23.62%	80 %- King PT et al 2006 (5)
Right side lesion in HRCT	48.81%	86%-Lynch DA et al 1999 (11)
AFB smear positive	3.15 %	3%- Bopaka RG et al 2015 (12)
Common organism isolated in sputum culture	P. aeruginosa 21%	H.influenza- lee JH et al 2004 (10)

In comparision it clearly states more incidence of bronchiectasis occur in late 40s.majority of patients visit physician with the complaints of Cough with purulent expectoration similarity was noted by Habesoglu MA et al (6) hence cough with prolonged duration should be suspicious of bronchiectasis. Among the spirometery pattern 43.3% had Obstructive pattern, this is due to airway inflammation and stasis of secretions, which are common in patients with bronchiectasis. Over use or inappropriate prescription of oral antibiotics without anti-pseudomonal activity could explain the predominance of P.aeruginosa in this study. Patients with cystic disease had a greater degree of functional impairment, compared to other types. This is in accordance with our study.

Limitation(s):

This study is subject to selection bias, since patients were selected from the inpatients of a single tertiary hospital. Therefore, the results might not represent the general population, probably including less severe or asymptomatic cases.

Conclusion:

Bronchiectasis might be one of the ongoing important reasons of mortality and morbidity, with worsening quality of life in that region. Commonly presenting by middle age the condition has typical symptoms and clinical findings. Postinfective causes such as pneumonia and tuberculosis appear to be the predominant etiology leading to bronchiectasis. This diagnosis should be actively considered in patients with a history of chronic cough with expectoration with or without hemoptysis, and/or breathlessness.

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Table 1- Age & Gender Wise Distribution of Study Subjects (N-127)

AGE CATEGORY (IN YEARS)	NUMBER OF PATIENTS (%)
14-25	2 (1.57 %)
26-35	11 (8.66%)
36-45	25 (19.68%)
46-55	62 (48.81%)
56-65	23 (18.11%)
>65	4 (3.14%)
Gender	
Male	55 (43.31%)
Female	72 (56.69%)

Table 2-Etiology For Bronchitis (N-127)

ETIOLOGY	NUMBER OF PATIENTS (%)
Pneumonia	10 (7.87%)
ABPA	3 (2.36%)
RRTI	12 (9.44%)
Idiopathic	54 (42.51%)
Tuberculosis	45 (35.43%)
Viral exanthem	4 (3.14%)

ABPA-Allergic Bronchopulmonary Aspergillosis, RRTI- Recurrent Lower Respiratory Tract Infections

Table 3-Spirometry & Radiological Pattern In Study Population (N-127)

SPIROMETRY	NUMBER OF PATIENTS (%)
Normal	52 (40.94%)
Obstructive	55 (43.30%)
Restrictive	3 (2.36%)
Mixed	17 (13.38%)
HIGH RESOLUTION CT	
CYSTIC	36 (28.34%)
CYLINDRICAL	79 (62.20%)
VARICOSE	2 (1.57%)
MIXED	10 (7.87%)

Table 4- Association Between Sputum Culture And Radiological Types Of Bronchiectasis In Study Patients

SPUTUM CULTURE	Number of patients	Cystic	Cylindrical	varicose	Mixed	p-value (Chi square test)
Pseudomonas aeruginosa	27 (21.25 %)	14	8	0	5	0.0004 (<0.05)
Hemophilus influenza	15 (11.81%)	1	13	1	0	0.0352 (<0.05)
Fungal hyphae	3 (2.36%)	3	0	0	0	0.0511 (>0.05)
Acienetobacter baumannii	5 (3.93%)	1	3	0	1	0.7564 (>0.05)
Eschericia coli	17 (13.38%)	1	12	1	3	0.0383 (<0.05)
Klebsiella pneumonia	17 (13.38%)	8	9	0	0	0.2079 (>0.05)
Mycobacterium abscessus	1 (0.78%)	1	0	0	0	0.4667 (>0.05)
Mycobacterium tuberculosis	3 (2.36%)	1	2	0	0	0.9549 (>0.05)
Streptococcus pneumonia	15 (11.81%)	5	9	0	1	0.9272 (>0.05)
Staphylococcus aureus	10 (7.87%)	1	9	0	0	0.3003 (>0.05)
Negative	14 (11.02%)	0	14	0	0	0.0227 (<0.05)

Original Article

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Physiology Section

Effect of Mint Flavoured Chewing Gum in Observing Changes in Cognitive Function while Assessing Test Performance- An Interventional Study

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BY-NC-ND

ABSTRACT

Introduction: Cognition is the mental process of acquiring knowledge and understanding through aspects such as awareness, perception, reasoning, memory and judgement. Chewing movement of jaw stimulates memory parts of brain by increasing blood flow and glucose delivery. Taste and odour of mint is also known to stimulate memory areas of the brain. The synergistic effect of chewing and flavour is expected to have a greater effect on cognition than chewing alone.

Aim: To assess the effect of use of mint flavoured, flavourless and absence of chewing gum on an individual's cognitive function among the medical undergraduates.

Materials and Methods: This comparative, interventional study, was conducted in the Department of Physiology at Velammal Medical College and Hospital, Madurai, Tamil Nadu, India, August 2019 to September 2019. Study involved 75 (39 females, 36 males) MBBS first year students, aged 18-20 years. Only students with cognitive score between 28-30 based on Mini-Mental State Exam (MMSE) score were included in the study and were divided into 3 groups. Group A (n=25) who were given mint flavoured chewing gum, Group B (n=25) given flavourless chewing gum and Group C (n=25) the control group, not provided with chewing gum. Baseline memory, Heart Rate (HR), Reaction Time (RT) and Stress Levels (SL) were recorded. Groups were taken into separate rooms where they were allowed to study a particular topic i.e Parkinson's disease for 30 minutes. Then they were allowed to take tests on standard Parkinson's questionnaire for 20 minutes and assessed based on the test performance. Group A and Group B were provided with chewing gums both during studying the topic as well as taking tests. Post intervention test performance (short term memory), HR, RT and SL were again recorded. Test performance was also assessed after one month to assess the effect of chewing gum on long term memory. One-way Analysis of Variance (ANOVA) and paired t-test were used to compare all the post test parameters between the three groups.

Results: A statistically significant increase in short term memory (p-value=0.001) and HR (p-value=0.001) were observed after intervention. Similarly, short term memory level of the three groups subjects statistically differed (p-value=0.001). When considering the reaction time (p-value=0.068) and stress level (p-value=0.927), there was no significant difference among the three groups after the intervention. Assessment of the test scores alone after one month (long term memory) showed a significantly higher score (p-value <0.001) in Group A when compared with the other two groups.

Conclusion: Mint flavoured chewing gum improves cognition as evidenced by improvement in test scores, alertness and attention. The performance in the flavour less chewing gum group was lesser than mint flavoured group, but significantly better than control group.

Keywords: Heart rate, Memory, Menthol, Reaction time

INTRODUCTION

Chewing gum is generally preferred for maintaining alertness and preventing sleepiness while studying or driving. It creates a sense of euphoria and helps rejuvenate especially flavoured ones. It tends to affect a range of cognitive functions including aspects of memory, selective and sustained attention, psychomotor speed and accuracy [1]. The chewing movement of jaw stimulates nerves and parts of brain leading to increase in cerebral blood flow [2]. This increased blood flow to brain enhances glucose delivery to memory associated regions improving both episodic and working memory [3]. Chewing also facilitates release of insulin which influences memory via central mechanism [4]. The process of mastication increases the sympathetic nervous system activity and decreases parasympathetic activity [5]. Chewing gums activates brain centres such as prefrontal cortex, middle frontal gyrus (Brodmann's area 9 and 46) in dorsolateral prefrontal cortex, right prefrontal motor cortex, precuneus, thalamus, hypothalamus and inferior parietal lobe which has an enhancing effect on memory [6]. Though the effect of chewing gum on cognition and memory was evaluated in previous studies, the results are still debatable [7-9].

Alertness refers to the ability to develop and sustain a state of mental readiness. Attention requires efficient perception, learning, memory, and reasoning. The parameters for measuring alertness and attention were heart rate and reaction time. Reaction time is the minimum time taken to respond to a stimulus and it helps in assessing the integrity of the nervous system. The effect of chewing produced changes in reaction time and Event Related Potential waveforms thereby improving the cognitive processing [10]. The role of chewing gum in altering alertness and attention has been proved in many previous studies [11,12]. Chewing of flavoured gum consistently increases the beta rhythm of electroencephalogram, which is supposed to be the rhythm associated with arousal and alertness [13]. However, few studies have also demonstrated the same arousal effect with flavourless and odourless chewing gum [6,10].

Stress induces the release of free radicals in the body and is considered to be the cause for various health related problems like atherosclerosis, endothelial damage, hypertension, asthma, irritable bowel syndrome, cancer [14]. Stress can impair the memory function [15]. The results of the previous studies on the effect of

chewing gum on stress were controversial [16,17]. Few studies had reported a reduction in stress level under acute social stress, whereas no reduction in anxiety was found after chewing gum in other studies [16,17].

Smell is perceived in widespread areas of the limbic system including hippocampus, entorhinal cortex, orbitofrontal cortex and amygdala. Perception of taste occurs in the insular cortex. Integration of smell and taste sensation happens in the orbitofrontal cortex. All the above mentioned areas are associated with learning and memory [18]. It is expected that mint flavoured gums have an arousing effect in these areas both via taste and smell modalities. Though researchers were unable to point exactly why chewing gum boosts memory, attention and cognitive reasoning skills, the results of different studies clearly showed that it does. Chewing gum has similar effect to that of strenuous activity which boosts test performances.

Hence, it was hypothesised that mint flavoured chewing gum can improve memory by increasing blood flow and stimulating memory areas of the brain which could lead to improved test performance. The aim of the present study was to determine the effect of mint flavoured chewing gum on short and long term memory, alertness, attention and stress among the medical undergraduates.

MATERIALS AND METHODS

This comparative, interventional study, was conducted in the Department of Physiology at Velammal Medical College and Hospital, Madurai, Tamil Nadu, India, August 2019 to September 2019, after obtaining Institutional Ethical Clearance (IEC No: VMCIEC/24/2019).

Sample size calculation: Sample size was calculated with Cohen's D effect size fixed as 0.49 with 95% confidence level and 80% power. The minimum sample size thus calculated was 16.

Inclusion criteria: The study was initially explained in detail to all 150 first year MBBS students. Willing 126 first year MBBS students in the age group of 18-20 years, of both the genders, were enrolled for the study.

Exclusion criteria: The students were then screened and those with dental problems (undergoing orthodontic treatment, having fixed appliances), refractive errors, ophthalmic lesions, common cold, having difficulty in mastication (temporomandibular joint issues), under medication were excluded from the study. Smokers and those who have the habit of chewing gums regularly (more than 6/week) were also excluded.

Study Procedure

After the initial screening, baseline cognitive functions of 91 students were assessed using MMSE tool and only those with scores between 28-30 were included in the study [19]. Total 16 students were excluded, as the score was less than 28.

After obtaining informed voluntary consent from the remaining 75 students, the participants were randomly divided into three groups, three types of paper lots were created using alphabets A, B and C of 25 each. All the 75 students were instructed to select one paper lot and based on that, they were grouped accordingly.

- Group A (n=25) was the mint flavoured chewing gum group,
- Group B (n=25) was the flavourless chewing gum group
- Group C (n=25) was the control group which were not provided with chewing gum.

Wrigleys extra long lasting flavour (sugar free) peppermint gum (Illinois, U.S) was used in the mint flavoured group and Wrigleys gum base (synthetic rubber) was used in the flavourless chewing gum group. Group A and B were the interventional groups where exposure (chewing gum) was assigned.

Description of Intervention

The subjects were instructed to refrain from caffeinated drinks (and other stimulants) and exercise on the morning of the test. The test was conducted between 8 am-1 pm in the Department of Physiology. Demographic and anthropometric data including age, gender, weight and height was collected from all the 75 participants. Informed written consent was obtained from each participant. They were allowed to relax for 5 minutes after which visual reaction time and heart rate were recorded for all the students. They were then instructed to fill the stress questionnaire. As the topic chosen for the study was Parkinson's disease, a 10 minutes introductory lecture on this topic was delivered to all the 75 participants simultaneously. The students listened without taking notes. It was also informed to all the participants priorly that they will be placed randomly in either groups.

After that, they were divided into groups and sent to three separate rooms. Now group A was given one piece of mint flavoured chewing gum and group B was given one piece of flavourless chewing gum. They were told to chew constantly while reading. Group C was not given chewing gum. The participants were instructed to read the Parkinson's disease topic from Comprehensive Textbook of Medical Physiology by G.K Pal, for 30 minutes [20]. After a resting period of 10 minutes, they were allowed to take tests for 20 minutes on the same topic using a standard questionnaire simultaneously [21]. Group A were provided again with one piece of mint flavoured gum and group B with one more piece of flavourless chewing gum while doing the test. Immediately after the test, while group A and group B were still chewing, visual reaction time and heart rate was recorded and the post stress questionnaire was filled. After a period of one month all the groups were intimidated to take the same test with the same set-up and the test performance results were analysed. During this one month interval, the participants were restricted from chewing gums. After one month, they were allowed to take chewing gum only during the test performance.

Data Collection Method and Tools

- **Measurement of reaction time** [22]

Visual Reaction Time (VRT) was measured with the help of discriminatory and choice reaction time apparatus (Anand Agencies, Pune). The VRT for light stimuli with an accuracy of 0.001 second was measured in the sitting posture in a quiet room [22]. To record the baseline VRT for light stimulus (red), initially the subject was instructed about the complete procedure. The subject was asked to keep pressing the response button of the visual stimulus using the index finger of right hand. He should remove his finger immediately after he sees the stimulus. The value of VRT in milliseconds was displayed on the screen. Sufficient time was given for the participants to get acquainted with the procedure thoroughly. After the practice trial, once the patient felt comfortable, three readings were taken and the fastest response value was taken as the final reaction time.

- **Heart rate**

The Heart rate was measured with the help of masimo pulse oximeter to assess alertness [23].

- **Stress**

The Stress was assessed with the help of perceived stress scale questionnaire [15]. This scale includes 10 questions and the scores:

0-13 is considered as low stress,

14-26 as moderate stress

27-40 as high stress.

The participants read the Parkinson's disease topic from the book Comprehensive Textbook of Medical Physiology by G.K Pal [20].

- **Short and Long term memory**

Both short term and long term memory was assessed using Parkinson's disease questionnaire [21]. This included 20 questions on definition, causes, features and treatment of Parkinsons disease. The present study used a modified version of this questionnaire including the same 20 questions but for a score of 20 marks. The questions were of open-ended text type and each correctly answered question carried one mark.

STATISTICAL ANALYSIS

The data was entered into Microsoft excel and analysed using Statistical Package for Social Sciences (SPSS) version 20.0. Descriptive statistics like mean and standard deviation were used to represent continuous variables. One-way Analysis of Variance (ANOVA) was used to compare more than three groups on the basis of mean and standard deviation scores. Bonferroni multiple comparison test was used to compare the combinations of two groups in ANOVA. Paired sample t-test was used to compare the scores of before and after intervention. A 5% level of significance was considered statistically significant (p-value <0.05).

RESULTS

To ensure that all the selected participants (based on MMSE score) had the same level of heart rate, reaction time and stress before the beginning of the test, baseline equality verification was done. Statistical analysis was done to ensure that baseline parameter values were similar for all the groups before intervention. The p-value was non significant (p-value >0.05) among all the three group subjects, showing same level of heart rate, reaction time and stress [Table/Fig-1].

After the intervention, three groups' subjects' heart rates significantly differed (p-value <0.001). Especially, Bonferroni test revealed that the heart rates of control group subjects (A vs C has p-value=0.001 and B vs C has p-value=0.001) significantly differed compared to that of subjects of the other two groups. Similarly, short term memory level of the three groups subjects statistically differed (p-value=0.001). When considering the reaction time (p-value=0.068) and stress level (p-value=0.927), there was no significant difference among the three groups after the intervention [Table/Fig-2].

After the intervention, there was a significant improvement in the heart rates in group A (p-value <0.001) whereas there was no significant change in the heart rates in group B (p-value=0.665). In addition, reaction time of the subjects who use mint flavoured chewing gum had significantly reduced after the intervention (p-value <0.01). Similarly, the reaction time group A and B had significantly reduced after the intervention (p-value=0.006, p-value=0.001, respectively). However, stress level of the subjects was not significantly changed after the intervention in all three groups [Table/Fig-3].

Memory scores levels differed significantly across various groups after the intervention. Mint flavoured chewing gum group had high memory score compared to other groups [Table/Fig-4].

Overall, there was a significant change among the three group subjects in terms of heart rates, short term and long term memory levels after the intervention (p-value=0.001). Therefore, the intervention had the effect on the heart rates, short term and long term memory levels.

Group A and B had the similar level of effect on heart rate (p-value=0.371). However, group A had more effect on short term memory level compared to that of group B (p-value=0.029) after the intervention. When considered the within the group variations after the intervention, subjects who used group A had significant improvement in the heart rates and reduction in the reaction time. Therefore, group A was better than group B in terms of significant improvement in heart rate, short term and long term memory levels.

Parameter	Group A Mean±SD (n=25)	Group B Mean±SD (n=25)	Group C Mean±SD (n=25)	F-Statistic	p-value
Heart rate (beats/min)	83.00±10.09	86.72±15.63	78.80±10.20	2.614	0.080
Reaction time (milli seconds)	212.36±37.05	219.96±29.02	198.32±33.97	2.683	0.075
Stress level	19.12±9.08	19.64±6.50	19.52±7.11	0.032	0.969

[Table/Fig-1]: Comparison of heart rate, reaction time and stress level among the three groups before intervention.
One-way ANOVA *p-value <0.05 was considered statistically significant

Parameters	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD	F-Statistic	p-value	Bonferroni, p-value		
						A vs B	A vs C	B vs C
Heart rate (beats/min)	93.36±10.55	88.20±15.63	76.08±7.46	14.342	0.001**	0.371	0.001	0.001
Reaction time (milli seconds)	193.84±29.04	197.44±23.77	178.16±37.52	2.799	0.068	1.000	0.224	0.088
Stress level	18.12±9.31	18.64±6.72	18.96±6.70	0.076	0.927	1.000	1.000	1.000
Short term memory level	13.80±5.80	10.54±3.63	7.40±3.09	13.600	0.001**	0.029	0.001	0.038

[Table/Fig-2]: Comparison of heart rate, reaction time, stress level and short term memory level among the three groups after intervention.
**p-value <0.01 will be considered statistically highly significant; STML: Short term memory level; One-way ANOVA, Bonferroni multiple comparison test

Parameters	Group A Mean±SD		Group B Mean±SD		Group C Mean±SD	
	Preintervention	Postintervention	Preintervention	Postintervention	Preintervention	Postintervention
Heart rate (beats/min)	83.00±10.09	93.36±10.55	86.72±15.63	88.20±15.63	78.80±10.20	76.08±7.46
	p-value=0.001**		p-value=0.665		p-value=0.021*	
Reaction time (milli seconds)	212.36±37.05	193.84±29.04	219.96±29.02	197.44±23.77	198.32±33.97	178.16±37.52
	p-value=0.006**		p-value=0.001**		p-value=0.002**	
Stress level	19.12±9.08	18.12±9.31	19.64±6.49	18.64±6.72	19.52±7.11	18.96±6.70
	p-value=0.244		p-value=0.223		p-value=0.265	

[Table/Fig-3]: Comparison of heart rate, reaction time and stress levels before and after the intervention within the groups while assessing the short term memory.
HR: Heart rate; RT: Reaction time; SL: Stress level; Paired sample t test; *p-value <0.05 will be considered statistically significant; **p-value <0.01 will be considered statistically highly significant

Group	N	Mean score of the test questionnaire (20 max)	Std. Deviation	F-value	p-value
Group A	25	8.2400	4.69734	8.681	0.001**
Group B	25	6.5800	2.64449		
Group C	25	4.0800	2.97097		

[Table/Fig-4]: Long term memory score after one month between the 3 groups.
**p-value <0.01 will be considered statistically highly significant; Statistical test used: one- way ANOVA

DISCUSSION

The act of mastication itself increases the blood flow to fronto-temporal cortex, caudate nucleus, thalamus, rolandic areas, insular cortex, cingulated gyrus and cerebellum as observed with xenon-enhanced computed tomography [24]. The temporomandibular joint movements due to chewing not only increases blood flow but also glucose delivery to mainly bilateral temporal cortical areas [2]. Since, the temporal cortex is associated with memory regions of the brain including hippocampus, activation of these areas occur. In the present study, test scores had increased significantly after chewing gum for 20 minutes in mint flavoured group indicating improvement in short term memory. Short term memory lasts for seconds to hours through processing mainly in hippocampus [25]. Since taste and smell centres are situated in the memory regions of the brain, chewing along with odour and taste of mint could have strongly activated memory areas of the brain improving the test scores in this group compared with the other two. This also explains the better performance in the flavourless chewing gum group compared with the non chewing control group. The results of the current study coincide with the results of the previous studies, where the test performance improved significantly in the gum chewing group when compared to the group which mimicked chewing movements and the group which did not chew gum [9,26]. But the present study results are contradictory to the results of a study done in 2008, where the chewing gum did not improve the short term memory performance scores [1].

Alertness and attention in the present study was checked by changes in heart rate and visual reaction time. A significant increase in heart rate was observed in mint flavoured group alone. This differs from the results of a chewing gum study where increase in heart rate was not observed, though there was increase in cortisol level and work performance [27]. The results of the present study is in accordance with many previous studies which had observed increase in heart rate due to chewing [1,9,27]. This was observed mainly during chewing and immediately after chewing. This increased heart rate by pumping more blood could have activated the memory regions of the brain. It could also be due to chewing associated increase in sympathetic activity and suppression of parasympathetic activity [4]. The decrease in heart rate in control group of present study could be due to increase in parasympathetic activity due to relaxation without any intervention.

In the present study, duration of visual reaction time decreased significantly within groups both in mint flavoured and in flavourless chewing gum group with no significant change between groups. This shows that an individual reacts faster to a visual stimulus due to the effect of mint flavoured chewing gum. The processing speed in brain had increased and this could be due to increased sympathetic activity and activation of ascending reticular activating system. The results of the present study coincide with the results of a previous study which showed quickened reaction time [12]. This quickening explains the increased activity in motor regions for alerting and executive networks especially anterior cingulate cortex and left frontal gyrus. Surprisingly reaction time significantly decreased in the control group and this could be due to the familiarity with the procedure when they did for the second time.

Stress scores did not change with chewing gum. In the present study, no stressful task was given to perform and post stress scores were assessed immediately after chewing gum for 20 minutes using a questionnaire. Regular gum chewing for 5 minutes, twice daily for 14 days had also resulted in significant decrease in stress level [8].

When all the three groups were assessed again with the same set of questions after a month for long-term memory, test scores were still significantly higher in mint flavoured group when compared with the other two groups. This could be due to stimulation of memory areas of the brain, mainly hippocampus, by the odour and taste of mint in the chewing gum. Hippocampus is essential for consolidating short term memory into long term memory and it was found that hippocampus is activated by mint flavoured chewing gum. The rate of chewing was not controlled and it was left to the choice of the participants as evidence indicates that more vigorous chewing does not modify the chewing effects on memory [27].

To confirm the effect of odour and taste in stimulating cognitive areas of the brain, a study was conducted by Hasegawa Y et al., where 25 healthy participants were divided into three groups-No taste/no odour chewing gum group, sweet taste/no odour gum group and sweet taste/lemon odour gum group. Cerebral blood flow was recorded during chewing using transcranial Doppler ultrasound and near infrared spectrometer while at the same time, bilateral masseter muscle activity was also monitored. Results revealed higher blood flow with sweet taste/lemon odour gum group compared to the other groups. This supports the additive role of both taste and odour in activating cognitive and motivational areas of the brain while chewing, than smell or taste alone [28]. A direct correlation was observed between peppermint oil aroma and improved memory by long term potentiation mechanism [29].

As it was recorded in a previous study that the effects of chewing gum started after 5 minutes of chewing and lasted for only 20 minutes, the present study participants were instructed to chew the gum for 20 minutes while studying and again for 20 minutes while doing the test [26].

Limitation(s)

The sample size was small. The results cannot be generalised as this study involved local medical students. Cross over between groups was not done. Heart rate and reaction time were not measured during long term memory assessment. Only subjective stress levels were assessed. Future studies may focus on measuring stress level after providing an exposure to acute stressor. Functional Magnetic Resonance Imaging (MRI) could be done to assess the changes in memory areas of brain. Further studies are also needed to study the long duration of gum chewing on memory.

CONCLUSION(S)

The present study results showed that mint flavoured chewing gum improved alertness and attention as shown by increase in heart rate and decrease in reaction time. Mint flavoured chewing gum improved memory as shown by the increase in test performance scores immediately as well as after one month. As participants chewed gum during learning and again during the test performance, their recall was improved by the taste and odour of mint which stimulated the memory areas of the brain. But the present study failed to show any improvement in stress level. Flavourless chewing gum improved memory, attention, and alertness when compared to the control group, without having any significant effect on stress level. Chewing mint flavoured gum before exams could help the younger generation perform better, as the amount of information to be processed and reproduced for students, especially medicos, is very huge. Mint flavoured chewing gums are cost-effective, easily accessible and can be chewed before the tests to improve cognitive function.

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