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COMPARATIVE EVALUATION OF SALIVARY AMINO ACIDS PROFILE AMONG YOUNG ADULTS

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Abstract

Aim: The aim of this study is to compare the salivary amino acids profile among young adults and identify any variations based on demographic factors and to conduct all basic amino acids profiling tests rapidly.

Introduction: Saliva is an easily accessible and non-invasive biofluid that offers valuable insights into nutritional status and overall health. Amino acids, the fundamental constituents of proteins, play vital roles in various physiological processes.

Methods: Saliva samples from 4 males and 4 females were collected The samples were placed in a freezer at -20° Celsius to this 0.15% triton x solution was added. 500 microlitres of 1 normality hcl was added to 100 microlitre sample. Ninhydrin analysis was done.

Results: It was observed that amino acid content in saliva was different for each sample.The total Amino acid content was more in females than in male.

Conclusion: This study provides valuable insights into nutritional status and health assessment for young adults using non-invasive salivary biomarkers. Further research and longitudinal studies are needed to validate these findings and their clinical implications.

Keywords: Salivary amino acids, Young adults, Nutritional assessment, Dietary habits, Non-invasive biomarkers, Health assessment.

INTRODUCTION

Saliva is a hypotonic mixture of oral mucosal exudates, gingival crevicular fluid, and salivary acini. The salivary glands, of which the parotid, submandibular, and sublingual glands are the principal ones, release around 90% of saliva[1].Salivary glands with high permeability are bordered by capillaries, blood, and acini and can exchange molecules. As a result, biomarkers in the bloodstream may enter acini and subsequently release into saliva. It has a pH between 6.6 and 7.1, a relative density between 1.004 and 1.009, and neither colour nor smell. Each day, the average human generates 600 mL of saliva. Saliva is 99% water while the remaining 1% is made up of organic molecules such as salivary amylase, mucopolysaccharide, mucin, and lysozymes as well as certain inorganic substances like Na+, K+, and other cations[2].

Saliva serves several functions. Firstly, it aids in mouth cleaning by washing away bacteria and food residues, keeping the breath fresh. Secondly, it contains salivary amylase, a type of enzyme that breaks down starch into maltose and occasionally glucose in the mouth[3]. Thirdly, lysozymes and thiocyanate ions in saliva have bactericidal properties, making it an essential component of the human body's nonspecific immune system. Lastly, saliva is secretory and can carry risk factors for certain diseases, as it may excrete or transmit substances like KI, Pb, Hg, and viruses such as rabies, polio, and human immunodeficiency virus[4].

In the past, doctors used serum or urine tests for disease diagnosis, which could be painful or embarrassing. Now, saliva is seen as a promising source of biological markers, encompassing biochemical changes, DNA, RNA, proteins, and microbiota structure[5]. Collecting saliva is relatively safe and reduces the risk of virus transmission. Therefore, saliva offers a new, non-invasive, and straightforward method for disease diagnosis, with the potential to replace serum or urine tests in the future[6].

Research on caries and oral cancer has shown intriguing findings regarding saliva. In the case of caries, studies have established a favorable correlation between the volume of Streptococcus mutans and Lactobacillus in saliva and the occurrence of dental caries[7]. In case of oral cancer, tumor-specific DNA somatic mutations detectable in saliva, plasma, or other body fluids are linked to the initiation and progression of malignancy. These mutations serve as valuable biomarkers for identifying oral and other tumors[8]. Notably, all patients with oral tumors tested positive for tumor-specific DNA in their saliva, while 47%–80% of patients with tumor-specific DNA in their saliva also had tumors elsewhere in the body[9]. These findings collectively underscore the significance of saliva in understanding and detecting both dental caries and oral cancer.

Pancreatic cancer presents a grim reality with a significant mortality rate, claiming the lives of 98% of diagnosed patients worldwide, where over 200,000 cases are recorded annually[10]. Saliva analysis has unveiled overexpression of genes hsa-miR-210 and let-7c in pancreatitis patients, while pancreatic cancer patients exhibit elevated levels of hsa-miR-21, hsa-miR-23a, hsa-miR-23b, miR-29c, and hsa-miR-216, some of which are also overexpressed in precursor lesions[11]. In the case of diabetes mellitus, saliva analysis has uncovered the potential of -2-macroglobulin levels as a valuable indicator of glycemic control in type 2 diabetes mellitus due to its positive correlation with HbA1c[12]. The strong correlation between HbA1c levels and salivary glucose concentrations offers individuals with diabetes mellitus a convenient means of monitoring their blood glucose levels using saliva[13].

The study "Comparative Evaluation of Salivary Amino Acids Profile Among Young Adults" is likely intended for diagnostic purposes. By analyzing the salivary amino acids profile of young adults, it can potentially serve as a non-invasive and convenient method for assessing their nutritional status and detecting any abnormalities related to amino acid metabolism. Such diagnostics could help identify underlying health conditions or nutritional deficiencies, leading to early interventions and personalized treatment plans for the individuals involved.

MATERIALS AND METHOD

Saliva samples from 4 males and 4 females of age group 18-23 years were collected. The samples containing 0.15% triton x were placed in a defreezer at -20° Celsius overnight and centrifuged at 16000 RPM for 15 mins. The supernatant was collected, and debris were removed. Triton x solution is added to avoid viral contamination of the sample. Add equal volume of one normality HCl in 500 micro litres of saliva sample. After 12 hrs of heating at 80 degrees Celsius, following procedure was followed. Take 0.1 ml of the sample and standard amino acid in the range of 0.1 - 1.0 micro mole and add 2.0 ml of ninhydrin reagent. Heat the test tubes, covered with glass marbles, in a boiling water bath for 15 min. Cool the tubes to room temperature and add 7 ml of diluent solution and mix well. Measure the colour developed against the reagent blank at 570 nm. The duration of the study was three months.

RESULTS

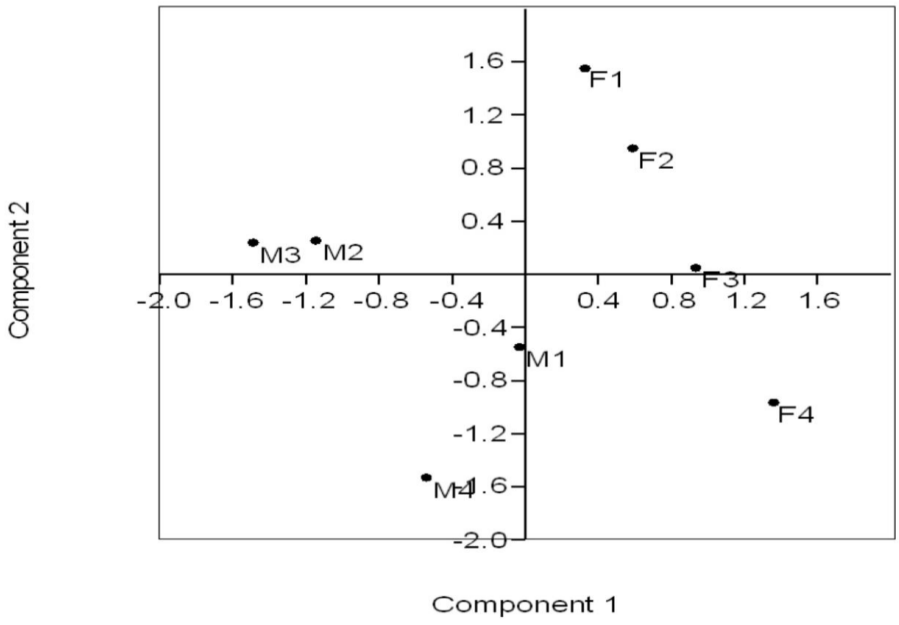

Fig 1. Principle co ordinate analysis of amino acid content among male and female saliva samples

Principal Common Coordinate analysis (Fig 1) shows dispersed view within the male and female groups. Thus, the saliva samples do not exhibit any common characteristics within gender categories. Similarly, it can also be safely concluded that each saliva sample exhibits a unique amino acid composition.

Amino Acids (µg/mL)	Male				Female			
	M1	M2	M3	M4	F1	F2	F3	F4
Aspartic acid	0.031	0.038	0.041	0.045	0.029	0.043	0.055	0.053
Glutamic acid	0.201	0.198	0.187	0.22	0.174	0.18	0.167	0.19
Serine	0.067	0.051	0.069	0.075	0.06	0.058	0.055	0.087
Histidine	0.203	0.191	0.185	0.188	0.184	0.192	0.2	0.21
Glycine	0.068	0.098	0.084	0.058	0.09	0.089	0.075	0.058
Threonine	0.155	0.158	0.161	0.158	0.166	0.165	0.159	0.169
Alanine	0.095	0.058	0.066	0.055	0.085	0.08	0.079	0.072
Arginine	0.058	0.047	0.045	0.051	0.062	0.06	0.068	0.059
Tyrosine	0.129	0.112	0.175	0.153	0.165	0.152	0.128	0.135
Valine	0.057	0.048	0.037	0.05	0.062	0.059	0.06	0.066
Methionine	0.028	0.03	0.032	0.025	0.038	0.04	0.029	0.025
Phenylalnine	0.037	0.011	0.013	0.028	0.045	0.041	0.046	0.05
Isoleucine	0.021	0.029	0.025	0.02	0.03	0.031	0.028	0.035
Leucine	0.025	0.02	0.021	0.029	0.021	0.03	0.027	0.025
Lysine	0.758	0.74	0.621	0.837	0.85	0.86	0.864	0.88

Table 1. Amino acid content of male and female groups from the age between 20-30 years

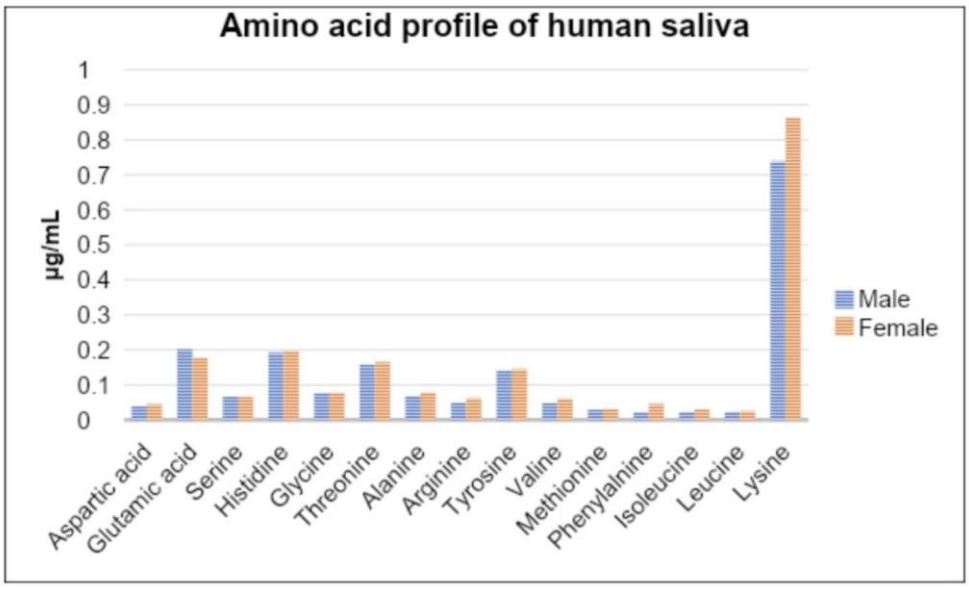

Fig 2. Amino acid profiling of human saliva from the age group between 20-30 years

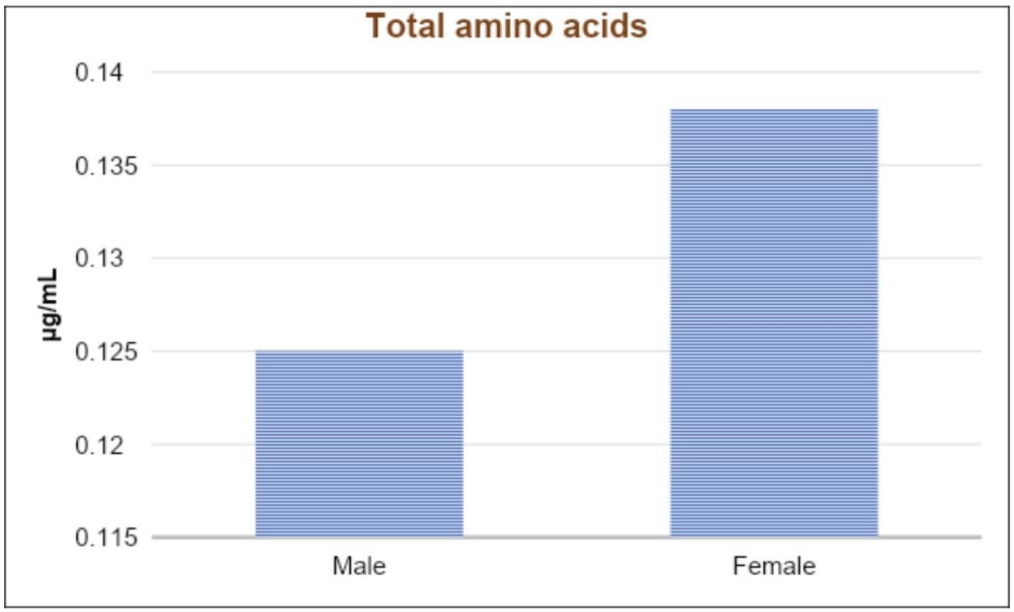

Fig 3. Total amino acids content in human saliva samples

From Table 1 and Figure 2, among the samples analyzed, both male and female samples exhibited a significantly very high proportion of Lysine while Glutamic acid, Histidine, Threonine were present in significant proportions (>0.15 µg / mL). Across gender comparison, Female groups exhibited a higher lysine content while males exhibited a higher Glutamic Acid component. Methionine, Phenylalanine, Leucine, Isoleucine and Aspartic Acid were found in lower proportion (<0.05 µg / mL) in both gender groups while females exhibited a higher proportion of these five amino acids compared to males. Also, the total Amino acid content was more in female than in male (Figure 3).

DISCUSSION

The current study demonstrates that ninhydrin analysis combined with ninhydrin analysis may accurately identify amino acids in spit samples. Due to complicated matrices, it is necessary to apply quick and accurate separation techniques when determining the presence of biomolecules in bodily fluids[14]. The main building blocks of muscles are called branched-chain amino acids (BCAAs), and research has shown that taking BCAAs can help to relieve muscle soreness. The significant problem of aging is thought to be resolved by BCAAs[15].

In the current study Glutamic acid levels are high in males when compared to females. glutamic acid has a variety of functions in both males and females. It affects memory, learning, and general brain function as a neurotransmitter in the central nervous system[16]. Additionally, it supports protein synthesis, which is essential for development, tissue repair, and maintenance. Both men and women can use glucose, which is produced from glutamic acid, as a source of energy for the body. Additionally, it supports the immune system by promoting the creation of immunological-related chemicals and antibodies, which are crucial for the body's ability to fight off infections[17]. It is essential for general metabolic processes that it participates in metabolic pathways, particularly the citric acid cycle. However, it's important to remember that high glutamic acid levels can cause excitotoxicity in the brain, which may be related to several neurological conditions[18].

The present study shows histidine levels are more or less equal in both males and females.An essential amino acid with important functions in the human body is histidine. It is crucial for immunological response and allergy reactions since one of its main roles is to act as a precursor for histamine, a neurotransmitter and immune system regulator[19]. Additionally essential for metal binding, histidine is a component of metalloproteins and participates in a number of enzymatic processes as well as acting as a coordination site for metal ions like iron and zinc[20]. Additionally, histidine plays a role in the synthesis of carnosine, a dipeptide with antioxidant characteristics that aids in the protection of cells from oxidative stress and supports the performance of muscles.

Threonine levels are higher in females than in males.The essential amino acid threonine has several important functions in the human body. It functions as a key building block for the production of proteins, which is necessary for tissue growth and repair[21]. Additionally, threonine helps to make collagen, which maintains the strength and integrity of bones and connective tissues. Its role in the generation of antibodies and immunoglobulins enhances the immune system's capacity to ward off infections[22]. Additionally involved in the manufacturing of neurotransmitters, threonine has an effect on cognition, mood, and brain activity. It contributes to fatty acid breakdown and energy synthesis in the context of metabolism([22,23].

Tyrosine levels in females are higher than males. A non-essential amino acid, tyrosine is crucial for several physiological activities. It acts as a precursor for the synthesis of a number of important neurotransmitters, including as dopamine, norepinephrine, and epinephrine, which are crucial for mood control, stress response, and general brain function[20]. The synthesis of thyroid hormones, which are essential for controlling metabolism and energy generation, is also aided by tyrosine. Additionally, it contributes to the synthesis of melanin, the pigment in charge of skin, hair, and eye color[24]. Tyrosine is significant for cognitive function, stress management, and maintaining general physiological balance, which highlights its importance in human health[25].

The amino acid analysis showed Lysine levels higher in females than in males. An important amino acid called lysine is crucial to many facets of human health. It is most known for its role in protein synthesis, which it plays in the development, maintenance, and repair of tissues and muscles[26]. Additionally, lysine is crucial for the absorption of calcium, which is necessary for maintaining healthy bones and a suitable bone density. Additionally, lysine has shown antiviral qualities and is occasionally taken as a supplement to control and stop herpes simplex virus outbreaks. The integrity of the epidermis, ligaments, and tendons is aided by its role in collagen production[27].

Analysis of squamous cell carcinoma cell line cultures suggests rapid membrane biosynthesis due to increased cell proliferation. In vitro studies using (2D) correlated spectroscopy revealed that a variety of metabolites, such as alanine, glutathione, histamine, isoleucine and leucine, were present at higher concentrations in OSCC patients than in controls. Salivary amino acids have provided to be a useful marker for the detection of ovulation during the menstrual cycle of women.

CONCLUSION

The amino acid composition of young individuals can be better understood through saliva analysis. Significant differences in salivary amino acid concentrations were identified and understood. implications for dietary assessment and potential connections to general health. Clinical applications and individualized treatments: The research opens up opportunities for clinical uses of salivary amino acid profile, such as the use of particular amino acid biomarkers as non-invasive diagnostic instruments or indicators of medical problems. Exploring individualized therapies based on salivary amino acid profiles can also result in more effective patient care through the development of specialized nutritional plans or the early detection of metabolic problems.

CONFLICT OF INTEREST

There are no conflict of interests.

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ETHICAL CLEARANCE

Not applicable for the current study.

REFERENCES

1. *Basilicata M, Pieri M, Marrone G, Nicolai E, Di Lauro M, Paolino V, et al. Saliva as Biomarker for Oral and Chronic Degenerative Non-Communicable Diseases. Metabolites [Internet]. 2023 Jul 27;13(8). Available from: http://dx.doi.org/10.3390/metabo13080889*
2. *Bagde H, Salam TA, Algharbi EM, AlSane MI, Almalki MM, Alshahrani RS, et al. Salivary Soluble CD44 Level in Chronic Periodontitis - A Comparative Assessment between Preoperative and Postoperative Scaling and Root Planing. J Pharm Bioallied Sci. 2023 Jul;15(Suppl 1):S508–12.*
3. *Meng L, Wang B, Wang B, Feng Q, Zhang S, Xiong Z, et al. Post-synthesis of a titanium-rich magnetic COF nanocomposite with flexible branched polymers for efficient enrichment of phosphopeptides from human saliva and serum. Analyst [Internet]. 2023 Aug 30; Available from: http://dx.doi.org/10.1039/d3an00989k*
4. *Di Gioia C, Santonico M, Zompanti A, Sabatini A, Grasso S, Ursini F, et al. Detecting Dementia by Saliva Analysis: A Fingerprinting Unobtrusive Method Based on a Fast and Cheap Sensor System. J Alzheimers Dis [Internet]. 2023 Aug 24; Available from: http://dx.doi.org/10.3233/JAD-230330*
5. *Lind PA, Siskind DJ, Hickie IB, Colodro-Conde L, Cross S, Parker R, et al. Preliminary results from the Australian Genetics of Bipolar Disorder Study: A nation-wide cohort. Aust N Z J Psychiatry. 2023 Sep 1;48674231195571.*
6. *Abraham D, Singh A, Goyal A. Symptomatic Irreversible Pulpitis induces increased levels of human NLRP3 in gingival crevicular fluid compared to Saliva- a case control observational study. J Endod [Internet]. 2023 Aug 25; Available from: http://dx.doi.org/10.1016/j.joen.2023.08.013*
7. *Thimmegowda U, Pai KS. Determination and correlation of matrix metalloproteases profile and total antioxidant capacity in severe early childhood caries children - A randomized clinical trial. J Indian Soc Pedod Prev Dent. 2023 Apr-Jun;41(2):104–10.*
8. *Diwan P, Nirwan M, Bahuguna M, Kumari SP, Wahlang J, Gupta RK. Evaluating Alterations of the Oral Microbiome and Its Link to Oral Cancer among Betel Quid Chewers: Prospecting Reversal through Probiotic Intervention. Pathogens [Internet]. 2023 Jul 30;12(8). Available from: http://dx.doi.org/10.3390/pathogens12080996*
9. *Zhou Y, Zeng H, Liu K, Pan H, Wang B, Zhu M, et al. Microbiota profiles in the saliva, cancerous tissues and its companion paracancerous tissues among Chinese patients with lung cancer. BMC Microbiol. 2023 Aug 28;23(1):237.*
10. *Sancho-Albero M, Sebastián V. Isolation of Extracellular Vesicles by a Microfluidic Platform to Diagnose and Monitor Pancreatic Cancer. Methods Mol Biol. 2023;2679:181–91.*
11. *Li Y, Feng Y, Yang Z, Zhou Z, Jiang D, Luo J. Untargeted metabolomics of saliva in pregnant women with and without gestational diabetes mellitus and healthy non-pregnant women. Front Cell Infect Microbiol. 2023 Jul 19;13:1206462.*
12. *Alagiriswamy AP, Nagaraj MG, Rajaram Mohan K, Narayanan M, Karunakaran P. Oral Manifestations of Type II Diabetes Mellitus and Comparison of Blood and Salivary Glucose Levels. Cureus. 2023 Jul;15(7):e42344.*
13. *Shin PK, Kim MS, Park SJ, Kwon DY, Kim MJ, Yang HJ, et al. A Traditional Korean Diet Alters the Expression of Circulating MicroRNAs Linked to Diabetes Mellitus in a Pilot Trial. Nutrients [Internet]. 2020 Aug 24;12(9). Available from: http://dx.doi.org/10.3390/nu12092558*
14. *Puerta A, Garcia-Lopez D, Tejedor-Matellanes P, Gomez-Ruiz L, de la Cruz-Rodriguez R, de Frutos M. Capillary gel electrophoresis of very high molecular weight glycoproteins. Commercial and tailor-made gels for analysis of human monomeric and secretory immunoglobulin A. J Chromatogr A. 2023 Jan 11;1688:463689.*

15. *Odessey R. Problems and Potential of Branched-chain Amino Acids in Physiology and Medicine. Elsevier Publishing Company; 1986. 368 p.*
16. *Yang C, Zhang Y, Zhang X, Tang P, Zheng T, Ran R, et al. An injectable, self-healing, and antioxidant collagen- and hyaluronic acid-based hydrogel mediated with gallic acid and dopamine for wound repair. Carbohydr Polym. 2023 Nov 15;320:121231.*
17. *Li Y, Luo X, Guo H, Bai J, Xiao Y, Fu Y, et al. Metabolomics and metatranscriptomics reveal the influence mechanism of endogenous microbe (Staphylococcus succinus) inoculation on the flavor of fermented chili pepper. Int J Food Microbiol. 2023 Aug 26;406:110371.*
18. *Holm J, Vanky F, Svedjeholm R. Glutamate infusion reduces myocardial dysfunction after coronary artery bypass grafting according to NT-proBNP: summary of two randomized controlled trials (GLUTAMICS I-II). Am J Clin Nutr [Internet]. 2023 Aug 30; Available from: http://dx.doi.org/10.1016/j.ajcnut.2023.08.012*
19. *Liu Y, Wang H, Liang Y, Guo Z, Qu L, Wang Y, et al. Dietary intakes of methionine, threonine, lysine, arginine and histidine increased risk of type 2 diabetes in Chinese population: does the mediation effect of obesity exist? BMC Public Health. 2023 Aug 15;23(1):1551.*
20. *Foratori-Junior GA, Le Guennec A, Fidalgo TK da S, Jarvis J, Mosquim V, Buzalaf MAR, et al. Comparison of the Metabolic Profile between Unstimulated and Stimulated Saliva Samples from Pregnant Women with/without Obesity and Periodontitis. J Pers Med [Internet]. 2023 Jul 11;13(7). Available from: http://dx.doi.org/10.3390/jpm13071123*
21. *Oishi K, Yajima Y, Yoshida Y, Hagihara H, Miyakawa T, Higo-Yamamoto S, et al. Metabolic profiles of saliva in male mouse models of chronic sleep disorders induced by psychophysiological stress. Sci Rep. 2023 Jul 10;13(1):11156.*
22. *Song Z, Fang S, Guo T, Wen Y, Liu Q, Jin Z. Microbiome and metabolome associated with white spot lesions in patients treated with clear aligners. Front Cell Infect Microbiol. 2023 Apr 4;13:1119616.*
23. *Tsagkari E, Deda O, Krokos A, Gika H, Papadopoulos MA, Chatzigianni A. Investigation of salivary biomarkers as indicators of skeletal and dental maturity in children. Orthod Craniofac Res. 2022 Nov;25(4):576–84.*
24. *Joering EA. The Relation of a Low Phenylalanine and Tyrosine Diet to Collagen Diseases. 1964. 110 p.*
25. *Wheeler DL, Yarden Y. Receptor Tyrosine Kinases: Structure, Functions and Role in Human Disease. Springer; 2014. 440 p.*
26. *Yan Q. Targeting Lysine Demethylases in Cancer and Other Human Diseases. Springer; 2023.*
27. *Wei D, Tian X, Zhu L, Wang H, Sun C. USP14 governs CYP2E1 to promote nonalcoholic fatty liver disease through deubiquitination and stabilization of HSP90AA1. Cell Death Dis. 2023 Aug 26;14(8):566.*
28. *Sneka S, Preetha Santhakumar. Antibacterial Activity of Selenium Nanoparticles extracted from Capparis decidua against Escherichia coli and Lactobacillus Species. Research Journal of Pharmacy and Technology. 2021; 14(8):4452-4. doi: 10.52711/0974-360X.2021.00773*
29. *Vishaka S, Sridevi G, Selvaraj J. An in vitro analysis on the antioxidant and anti-diabetic properties of Kaempferia galanga rhizome using different solvent systems. J Adv Pharm Technol Res. 2022 Dec;13(Suppl 2):S505-S509. doi: 10.4103/japtr.japtr_189_22.*
30. *Sankar S. In silico design of a multi-epitope Chimera from Aedes aegypti salivary proteins OBP 22 and OBP 10: A promising candidate vaccine. J Vector Borne Dis. 2022 Oct-Dec;59(4):327-336. doi: 10.4103/0972-9062.353271.*
31. *Devi SK, Paramasivam A, Girija ASS, Priyadharsini JV. Decoding The Genetic Alterations In Cytochrome P450 Family 3 Genes And Its Association With HNSCC. Gulf J Oncolog. 2021 Sep;1(37):36-41.*
32. *Sneka S, Preetha Santhakumar. Antibacterial Activity of Selenium Nanoparticles extracted from Capparis decidua against Escherichia coli and Lactobacillus Species. Research Journal of Pharmacy and Technology. 2021; 14(8):4452-4. doi: 10.52711/0974-360X.2021.00773*
33. *Vishaka S, Sridevi G, Selvaraj J. An in vitro analysis on the antioxidant and anti-diabetic properties of Kaempferia galanga rhizome using different solvent systems. J Adv Pharm Technol Res. 2022 Dec;13(Suppl 2):S505-S509. doi: 10.4103/japtr.japtr_189_22.*
34. *Sankar S. In silico design of a multi-epitope Chimera from Aedes aegypti salivary proteins OBP 22 and OBP 10: A promising candidate vaccine. J Vector Borne Dis. 2022 Oct-Dec;59(4):327-336. doi: 10.4103/0972-9062.353271.*
35. *Devi SK, Paramasivam A, Girija ASS, Priyadharsini JV. Decoding The Genetic Alterations In Cytochrome P450 Family 3 Genes And Its Association With HNSCC. Gulf J Oncolog. 2021 Sep;1(37):36-41.*