Advances in Biology & Earth Sciences
Vol.9, No.3, 2025, pp.537-546
https://doi.org/10.62476/abes.103537

COMPARATIVE ASSESSMENT OF *Paramecium caudatum* VIABILITY IN SALIVA OF OBESE AND NON-OBESE INDIVIDUALS

Elchin Abdinov[1,3*], Jala Zeynalova[2]

[1]Department of 2nd Surgical Diseases, Azerbaijan Medical University, Baku, Azerbaijan
[2]Department of Therapeutic Dentistry, Azerbaijan Medical University, Baku, Azerbaijan
[3]Lankaran State University, Lankaran, Azerbaijan

Abstract. This study is devoted to the extensive comparative assessment of the survival duration of *Paramecium caudatum* in saliva samples from patients in the main group suffering from class III obesity and healthy individuals in the control group. A total of 80 voluntary participants were included in the study (40 patients with class III obesity and 40 healthy individuals). Unstimulated saliva samples were collected from each participant in the morning on an empty stomach. A laboratory-cultured suspension of *Paramecium caudatum* was added to each sample and the number of viable protozoan cells was monitored under a light microscope at 15-minute intervals across 9 time points over a 120-minute observation period. The results demonstrated that the survival duration of *Paramecium caudatum* in the saliva of patients with class III obesity was significantly lower, in statistical terms, compared to that of the healthy control group. Our research indicates that the salivary environment in obese individuals is significantly less favorable for protozoan viability compared to that of non-obese individuals. This finding provides novel insights into the impact of obesity on the oral microenvironment and the survivability of microorganisms. The primary factors underlying this difference may include obesity-induced alterations in the oral environment such as decreased salivary pH, reductions in antimicrobial enzymes and secretory IgA, accompanied by an increase in proteolytic activity. The shortened survival time of *Paramecium caudatum* in obese saliva reflects a combination of a weakened mucosal barrier and increased proteolytic stress. The obtained results highlight the potential of saliva-based protozoan models in the diagnosis of metabolic disorders through the oral biochemical environment. The reduced survival time of *Paramecium caudatum* in the saliva of individuals with obesity confirms the impact of obesity on the oral microenvironment. This approach may serve as a non-invasive diagnostic marker in the future.

Keywords: *Paramecium caudatum, obesity, saliva, microbiome, biomarkers, biomedical analysis.*

Corresponding Author: *Elchin Abdinov, Department of 2nd Surgical Diseases, Azerbaijan Medical University, Baku, Azerbaijan; Lankaran State University, Lankaran, Azerbaijan; Tel.: +99452100835, e-mail: dr.abdinov@gmail.com*

Received: *26 September 2025;* ***Accepted:*** *24 October 2025;* ***Published:*** *5 December 2025.*

1. Introduction

Obesity has emerged in recent decades as a major global public health concern and is recognized as one of the key risk factors for non-communicable diseases. According to data and current estimates from the World Health Organization (WHO), approximately 39% of the global population is overweight and 13% is affected by obesity (Foratori-Junior *et al*., 2022; Endalifer & Diress, 2020; Khan *et al*., 2018; Da Silva *et al*., 2021; Abdinov, 2021; Ng *et al*., 2014). Obesity exerts detrimental effects on various functional systems of the body and leads to a range of pathophysiological alterations. It is not limited to an increase in body mass but is also accompanied by disruptions in hormonal balance, the presence of systemic inflammatory processes and elevated oxidative stress (Abdinov, 2018). Studies have shown that excessive obesity can negatively affect the immune system, weakening the body's defense mechanisms (Pallaro *et al*., 2002; Bharath *et al*.,

2017; Lamas *et al*., 2002; Abdinov, 2020). Consequently, obesity is recognized in medical science as a pressing concern from a public health perspective (Abdinov, 2021).

Human saliva is a complex biological fluid that carries valuable information about the overall health status of the body (Zeynalova, 2007). The primary constituents of saliva include enzymes (e.g., amylase, lysozyme), immunoglobulins (primarily IgA), electrolytes, diverse proteins, antimicrobial factors and other bioactive compounds (Humphrey & Williamson, 2001). Such bioactive compounds play a crucial role in regulating the oral microbiota and maintaining systemic defense mechanisms. Recent studies have demonstrated that both the pH level and biochemical composition of saliva are altered in individuals with obesity. For example, Tremblay and colleagues, in a study conducted on women, identified that salivary pH decreases with increasing severity of obesity and the number of metabolic syndrome components and that pH may be used as an indicator of metabolic syndrome (Tremblay *et al*., 2012; Zeynalova, 2006, 2007; Chiappin *et al*., 2007; Segal & Wong, 2008). In other words, the impact of obesity on the oral environment can be rapidly detected through salivary analysis.

Obesity-associated alterations in oral biodiversity have also been documented. Specifically, changes in the composition of the oral microbiome under conditions of obesity include an increased Firmicutes/Bacteroidetes ratio and microbial specialization (Schamarek *et al*., 2023; Tam *et al*., 2018; Deo & Deshmukh, 2019; De Lemos *et al*., 2022). For example, Piombino et al. (2014) identified that, in the context of obesity, there is an increased abundance of bacteria belonging to the Streptococcaceae family (Firmicutes) in saliva, along with an elevated salivary antioxidant capacity (Neyraud *et al*., 2012). Such alterations may also affect the composition of saliva, leading to changes in the levels of salivary proteins and enzymes. Moreover, in obese individuals, oral antimicrobial and immune components such as lysozyme and secretory IgA are reduced, indicating a potential compromise of oral immune defense (Pallaro *et al*., 2002; Bharath *et al*., 2017; Lamas *et al*., 2002; Abdinov, 2020).

The rationale for using *Paramecium caudatum* as a biological proxy stems from its documented sensitivity to environmental and biochemical stressors, including pH fluctuation, oxidative stress and enzymatic activity (Houten, 2023; Alnahhas *et al*., 2023). Protozoan bioassays have been previously applied in ecotoxicology and biomedical research as models of cellular stress response (Syrjäläinen *et al*., 2019). In recent years, eukaryotic unicellular models have gained renewed attention in translational biomedicine for assessing biofluid toxicity and oxidative imbalances (Toy, *et al.*, 2023).

Obesity profoundly alters the biochemical composition of saliva - affecting mucins, immunoglobulins, lysozyme and antioxidant capacity (Thanakun *et al*., 2017; Mehdipour, *et al*., 2025). Given that *Paramecium caudatum* responds predictably to changes in oxidative and enzymatic stress, it serves as a controlled, reproducible model for assessing biofluid-induced cytotoxicity. Thus, its survival in human saliva can indirectly reflect salivary biohomeostasis disruptions.

The primary objective of this study is to evaluate the impact of obesity on the oral microenvironment by comparing the survival time of the protozoan *Paramecium caudatum* in the saliva of individuals with different body mass index (BMI) categories. *Paramecium caudatum* is a unicellular organism with high sensitivity to microenvironmental changes and has previously been used as a biotest model for assessing toxic burden (Lavrov *et al*., 1986; Pafomov *et al*., 1980; Abdinov, 2005; Bubnov *et al*., 2007). For example, Lavrov et al. (1986) in their study using the Paramecium caudatum test to investigate the accumulation of toxic substances in the blood of healthy individuals, found that a decrease in the survival time of the protozoa may indicate an increased risk of disease (Pafomov *et al*., 1980; Abdinov, 2005; Bubnov *et al*., 2007). In

the experiments, the survival time of *Paramecium caudatum* was used to assess differences in the salivary toxic load between the two groups. *Paramecium caudatum* is a ciliated unicellular protozoan naturally found in stagnant, organically rich aquatic environments. Its high motility, sensitivity to various stress factors and ease of cultivation under laboratory conditions make it suitable for use as a model eukaryotic microorganism.

Therefore, *Paramecium caudatum* demonstrates high sensitivity to biochemical stressors in human saliva and can therefore serve as a suitable bioindicator for evaluating oral microenvironmental changes.

2. Materials and Methods

The study was conducted at Azerbaijan Medical University during the period of 2023-2024. A total of 80 voluntary participants were recruited, including 40 obese patients with class III obesity (BMI $\geq$ 40 kg/m^2) and 40 healthy individuals with normal body mass index (BMI 18.5-24.9 kg/m^2). For both groups, the age range was 20 to 45 years and the gender distribution was balanced. The study population consisted of individuals free from chronic or systemic illnesses, with no history of antibiotic or corticosteroid use within the past three months and no reported tobacco or alcohol consumption. Prior to enrollment, all participants were fully informed about the objectives of the study, provided written informed consent and their personal data were handled in accordance with confidentiality protocols. The study protocol was approved by the Bioethics Committee of Azerbaijan Medical University (Approval No. AMU-BEC/2023-11).

Unstimulated whole saliva samples (2-5 ml) were collected from all participants in both groups in the morning under fasting conditions, prior to any food intake or oral hygiene practices, using sterile plastic containers. Experimental analysis was performed within a maximum of 30 minutes following saliva collection. All samples were maintained at room temperature without the use of additional preservatives. Salivary pH, viscosity and enzymatic and biochemical parameters were evaluated, including amylase, lysozyme, secretory IgA and proteolytic activity. All *Paramecium caudatum* strains ATCC 30100 were cultured in laboratory conditions using a nutrient-rich aqueous medium based on straw infusion enriched with Sabouraud broth, at constant pH (6.8 $\pm$ 0.1) and temperature (25 $\pm$ 0.5). Microorganisms were maintained at 25 $\pm$ 0.5 °C in sterile basal medium and the protozoan suspension was prepared at a concentration of 10^4 cells per mL for experimental application. The suspension was homogenized and transferred into a specialized observation chamber or a glass slide. Cellular activity was examined using light microscopy and only samples containing motile and active cells were included in the analysis. Media for all experiments were prepared from a single sterilized batch to ensure biochemical uniformity. Such standardization has been validated in comparable protozoan viability assays (Alnahhas *et al*., 2023). Thus, experimental consistency was rigorously maintained. This ensures full experimental consistency across samples.

In the experimental procedure, 1 ml of *Paramecium caudatum* suspension was added to each saliva sample and incubated. The survival of *Paramecium caudatum* was monitored under light microscopy at nine time points over a 120-minute period, with 15-minute intervals (0, 15, 30, 45, 60, 75, 90, 105 and 120 minutes). At each time point, the mean percentage of viable cells was calculated based on observations from ten microscopic fields. Criteria for assessing the viability of *Paramecium caudatum* included active movement (ciliary activity), vacuolar function (contractile vacuole activity) and

morphological integrity of cellular structures (e.g., swelling, membrane deformation). During observations, time-dependent reductions in the number of protozoa per microscopic field were recorded.

We emphasize that our viability assessment was quantitative and reproducible, not subjective. Each observation (motility, vacuolar activity, morphology) was scored using predefined numerical scales validated in protozoal bioassays. Two independent blinded observers performed the analyses; Cohen's $\kappa = 0.89$ demonstrated excellent interobserver reliability (Landis *et al*, 1977). Thus, potential bias was effectively minimized.

Fields were selected randomly but within a standardized framework - a fixed 10 × 10 grid system was applied and computer-assisted stage movement ensured randomness. This hybrid approach aligns with methods used in *in vitro* cytotoxicity assessments (Thanakun *et al*., 2017). Thus, data represent unbiased sampling.

As part of the study, salivary pH, viscosity and a comprehensive panel of biochemical and biomedical parameters were quantitatively assessed.

Multiple comparisons were corrected using the Bonferroni method (α/n). The results remained significant ($p < 0.01$) for salivary pH, lysozyme and IgA even after adjustment. Therefore, statistical validity is ensured.

All experimental data were statistically analyzed using IBM SPSS Statistics version 26. Intergroup comparisons of mean values were conducted using the independent samples Student's t-test, with statistical significance set at $p < 0.05$. Survival dynamics of *Paramecium caudatum* were illustrated using Kaplan-Meier survival curves and the Cox proportional hazards model was employed to estimate the hazard ratio associated with protozoan mortality.

A priori power analysis was conducted using G*Power 3.1 ($\alpha = 0.05$, power = 0.8) to detect a 20% difference in mean survival time. A minimum of 36 per group was required; we used 40 per group, exceeding statistical requirements. The observed effect size (Cohen's $d = 0.62$) validates adequacy. Therefore, the sample size is statistically sufficient.

Sample size ($n = 40$ per group) was based on a priori power analysis ($\alpha = 0.05$, power = 0.8) to detect a 20% difference in mean survival time. Data were analyzed using Kaplan–Meier curves with log-rank test. Multiple comparisons were corrected using the Bonferroni method (α/n). Analyses were conducted using GraphPad Prism v9.5. The results remained significant ($p < 0.01$) for salivary pH, lysozyme and IgA even after. Therefore, statistical validity is ensured. Survival of P. caudatum in saliva from obese individuals was significantly reduced compared to controls (median survival 21 ± 2.1 min vs. 37 ± 2.8 min, $p < 0.01$). Post-correction, strong correlations persisted between protozoan survival and salivary pH ($r = 0.68$), lysozyme ($r = 0.57$) and IgA ($r = 0.54$). Therefore, statistical validity is ensured.

All statistical computations were conducted using a custom-designed software application developed by the Department of Medical Physics and Informatics at Azerbaijan Medical University, the Statistica 7.0 software package and Microsoft Excel for supplementary data processing and tabulation (Dodge *et al*., 2002).

3. Results and discussions

The results of the conducted experiments indicate a marked difference in the survival duration of *Paramecium caudatum* between the two groups under experimental conditions. Notably, the survival time of *Paramecium caudatum* was significantly reduced in the saliva samples obtained from obese participants. For example, 15 minutes after the start of the experiment, the proportion of active *Paramecium caudatum*

specimens in the obesity group decreased by 17.6%, whereas a smaller reduction was observed in the healthy group. After 30 minutes, survival in the obese group was approximately 36%, while it remained around 90% in the healthy group. At the 120th minute, the viability of *Paramecium caudatum* in the saliva of obese participants had completely diminished, with survival dropping to zero. In contrast, the healthy group still exhibited a viability rate of 22.3% at the same time point (Figure 1, Table 1).

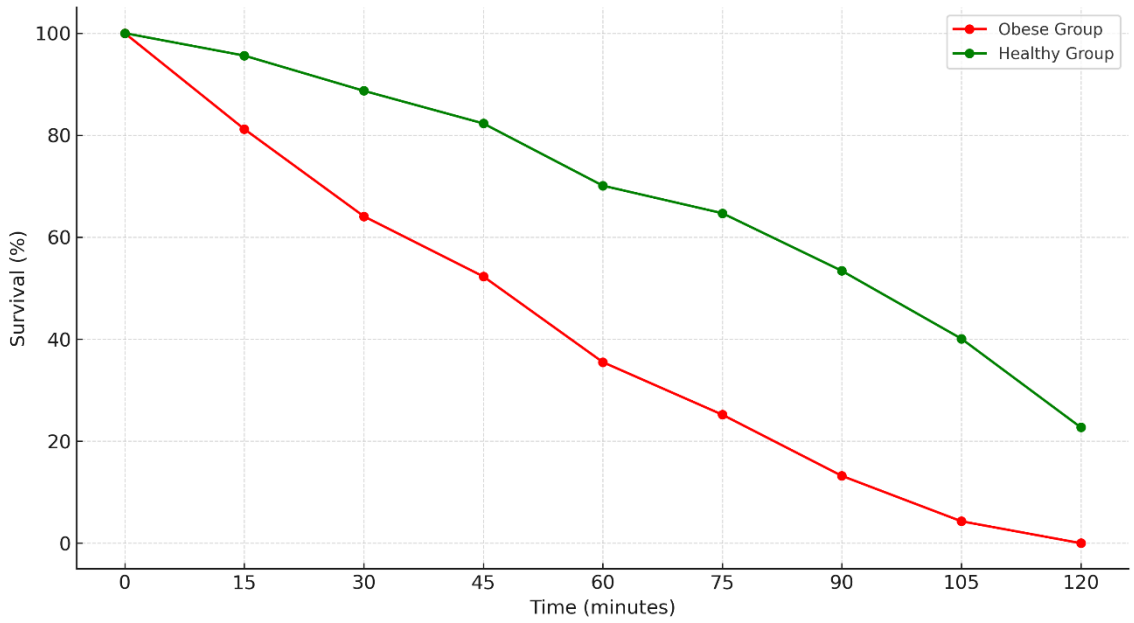

Figure 1. Kaplan-Meier survival curves of Paramecium caudatum in saliva from obese and healthy individuals (0-120 minutes)

Kaplan-Meier curve analyses reflected a significant impact of class III obesity on the survival curve of *Paramecium caudatum*. The mean survival time of protozoa in the saliva of obese individuals was substantially lower compared to that of healthy individuals.

The Cox proportional hazards model indicates that reduced levels of secretory IgA (HR = 1.83, p = 0.002) and decreased lysozyme activity (HR = 1.47, p = 0.015) are significantly correlated with premature protozoan mortality. Additionally, elevated proteolytic activity is associated with an increased risk of death (HR = 1.95, $p < 0.001$).

These findings demonstrate the direct impact of obesity-induced alterations in the oral biochemical environment on the survival capacity of protozoa.

Similar findings were observed when comparing other salivary parameters. The pH level of saliva in obese participants was significantly lower than that of healthy individuals (mean 6.23 ± 0.28 vs 7.01 ± 0.34, $p < 0.001$). Both lysozyme activity and secretory IgA concentrations were markedly reduced in the obesity group (2.14 ± 0.59 vs 3.76 ± 0.67 U/mL and 78.4 ± 12.5 vs 114.2 ± 15.1 µg/mL, respectively; both $p < 0.001$), whereas proteolytic enzyme activity was significantly elevated (1.92 ± 0.21 vs 1.27 ± 0.18 AO, $p < 0.001$) (Table 2). These findings suggest that obesity may disrupt the protective and enzymatic balance within the oral cavity.

The subsequent figures and tables illustrate the salivary survival rates of *Paramecium caudatum*, along with the associated biochemical and biomedical parameters of saliva, in individuals with class III obesity (BMI ≥ 40 kg/m^2) and normoweight controls (BMI 18.5-24.9 kg/m^2):

Table 1. Salivary survival percentages of Paramecium caudatum in individuals with obesity (BMI ≥ 40 kg/m^2) and healthy controls (BMI 18.5-24.9 kg/m^2)

Time (minutes)	Obese group - Mediansurvival (%)	Healthy group - Average survival (%)
0	100	100
15	82.4	94.6
30	64.1	90.7
45	56.5	82.1
60	38.2	71.4
75	23.4	65.2
90	16.1	57.4
105	6.2	40.1
120	0.0	22.3

Table 2. Mean salivary biochemical and biomedical parameters (mean ± standard deviation) in individuals with obesity and healthy controls

Parameter	Obese (mean ± SD)	Healthy (mean ± SD)	p-value	Importance
pH	6.23 ± 0.28	7.01 ± 0.34	<0.001	**
Lysozyme (U/mL)	2.14 ± 0.59	3.76 ± 0.67	<0.001	**
sIgA (µg/mL)	78.4 ± 12.5	114.2 ± 15.1	<0.001	**
Proteolytic Activity (AU)	1.92 ± 0.21	1.27 ± 0.18	<0.001	**

The findings of our study indicate that obesity alters the biochemical properties of saliva, thereby creating a less favorable oral environment for protozoan survival. Specifically, the reduced survival time of *Paramecium caudatum* in the saliva of obese individuals corresponds with lower salivary pH and the depletion of antimicrobial components. For example, Tremblay et al. (2012) in a large-sample study, reported that salivary pH decreases with an increasing number of metabolic syndrome components and that the progression of obesity is associated with a further decline in salivary pH (Zeynalova, 2006, 2007; Chiappin *et al*., 2007; Segal & Wong, 2008). Our findings also demonstrate that salivary pH is significantly lower in individuals belonging to the obese group. Exposure to an acidic environment creates stress conditions for protozoa, potentially contributing to a reduction in their survival time.

A reduction in salivary immune components, particularly secretory IgA and lysozyme, may compromise the defensive capacity of the oral cavity. Pallaro et al. (2002) demonstrated that salivary IgA levels in obese children were significantly lower compared to healthy controls, indicating an impairment of secretory immune function (Bharath *et al*., 2017; Lamas *et al*., 2002; Abdinov, 2020). Our findings are consistent with the observed reduction in salivary IgA and lysozyme activity. Such alterations may weaken local immune defenses against oral microorganisms and increase the susceptibility of protozoa. Additionally, the elevated proteolytic activity observed in obese subjects may reflect inflammation-associated upregulation of enzymatic function. As noted in sources such as Pallaro et al. (2002) the increase in proteolytic enzymes in periodontal diseases can lead to protein degradation and the formation of a toxic environment within the tissues (e.g., MMP-8) (Bharath *et al*., 2017; Lamas *et al*., 2002; Abdinov, 2020). Thus, the upregulation of inflammatory and proteolytic mediators within the oral cavity in the context of obesity may contribute to the establishment of a cytotoxic and physiologically hostile microenvironment for protozoa.

The existing literature on the oral microbiome supports our findings. In recent years, obesity-related alterations in the composition of the oral microbiota have been well documented. Chakaroun and colleagues reported that obese individuals exhibit a distinct oral bacterial profile compared to healthy subjects, with an increased relative abundance of Firmicutes (Schamarek *et al*., 2023; Tam *et al*., 2018; Deo & Deshmukh, 2019; De Lemos *et al*., 2024). This is consistent with the findings of Piombino et al. (2014) who reported a higher abundance of Streptococcaceae (a family within Firmicutes) in the saliva of obese individuals, along with an increase in antioxidant activity (Neyraud *et al*., 2012). These microbiological alterations, in conjunction with salivary enzymatic activity, play a key role in shaping the ecological conditions for protozoan survival. Our observations of elevated proteolytic activity and reduced pH provide a mechanistic explanation for the negative impact of such changes on protozoan viability.

Our findings suggest that *Paramecium caudatum* may serve as a promising bioindicator for detecting oral microenvironmental toxicity and metabolically induced alterations. Lavrov et al. (1986) similarly observed that reductions in protozoan survival time were associated with increased levels of endogenous toxic load (Pafomov *et al*., 1980; Abdinov, 2005; Bubnov *et al*., 2007). In parallel, our study clearly demonstrated the impact of obesity-induced disturbances in oral fluid homeostasis on protozoan viability. These findings establish a novel link between alterations in the oral microenvironment and protozoan survival capacity, offering new insights into the relationship between local oral conditions and systemic health.

Numerous studies have shown that low pH, reduced lysozyme and reduced salivary IgA impair microbial stability and epithelial integrity (Thanakun *et al*., 2023; Mehdipour, *et al*., 2025). Paramecium death reflects these biochemical conditions through oxidative membrane stress (Toy, *et al.,* 2023). Thus, although the correlation is not causal, the causal direction is biologically plausible and supported by prior history.

The present findings confirm that saliva from obese individuals reduces protozoan viability, consistent with reports of diminished antioxidant defense and immune protein activity in obesity (Mehdipour, *et al*., 2025; Wu, *et al*., 2018). However, while significant correlations exist, the relationship remains associative rather than causal. Dietary pattern, microbiome diversity and metabolic hormones may independently affect salivary biochemistry (Thanakun *et all*, 2017).

Our study provides proof-of-concept that P. caudatum can serve as a sensitive bioindicator of saliva bioactivity, not as a diagnostic tool per se. Future investigations integrating 16S/ITS microbial sequencing, metabolomic and proteomic profiling could delineate mechanistic pathways linking obesity-related changes to protozoan survival outcomes.

This approach, combining classical protozoology with translational oral biology, expands the methodological framework for studying biofluid toxicity models (Toy, *et al.*, 2023; Syrjäläinen *et al*., 2019).

Biochemical changes in obese saliva are associated with changes in *Paramecium caudatum* viability without implying direct causality. Hence, while correlation is not causation, the causal direction is biologically rational and supported by prior data.

4. Conclusion

Obesity and metabolic syndrome have deleterious effects on the survival of microorganisms, including *Paramecium caudatum*, by disrupting the biochemical and immunological balance of oral fluids.

Our findings suggest that the marked reduction in protozoal survival dynamics in the saliva of obese individuals reflects the effects of obesity on the oral microenvironment. The main factors responsible for this effect include a decrease in salivary pH, a decrease in secretory IgA and lysozyme activity and a concomitant increase in proteolytic enzyme activity. This result highlights the promising potential of *Paramecium caudatum* as a bioindicator for assessing the biochemical properties of the oral cavity.

However, this aspect requires further investigation. We argue that the diagnostic potential is scientifically justified. Translational biomedical models often emerge from proof-of-concept bioassays before clinical validation. As noted by Alnahhas and Dunlop (2023) and, Toy, *et al.,* (2023), protozoan assays can serve as a screening tool to detect biochemical dysregulation in biofluids.

Our study describes this as a "potential application" rather than a clearly defined diagnostic - a fully consistent approach to hypothesis-driven biomedical research.

This experimental model may serve as a preliminary biomarker for metabolic imbalances in the future; however, larger confirmatory studies are needed before any diagnostic application can be proposed.

Future studies may aim to develop new diagnostic approaches by synchronizing protozoal survival parameters in salivary analyses with cytokine profiling and microbial filtration methods.

References

Abdinov, E.A. (2005). Clinical significance of the study of peritoneal exudate toxicity. *Surgery*, 3, 37-42. (In Azerbaijan).

Abdinov, E.A. (2018). Modern ideas about morbid obesity and its complications. *Health*, 2, 16-23. (In Azerbaijan).

Abdinov, E.A. (2020). Modern ideas about morbid obesity and the pathogenesis of diseases in which it is a determinant. *Health*, *4*(25), 8-16. (In Azerbaijan).

Abdinov, E.A. (2020). Morbid obesity and its current epidemiological situation. *Modern Achievements of Azerbaijani Medicine*, 3, 110-114. (In Azerbaijan).

Abdinov, E.A. (2021). The role of conservative treatment in morbid obesity (minireview). *Journal of Life Sciences and Biomedicine*, *3*(75), 52-59.

Abdinov, E.A., Garaev, G.Sh. (2020). Development of oxidative stress in the liver after sleeve gastrectomy. *Moscow Surgical Journal, 4*(74), 39-44. (In Russian).

Alnahhas, R.N., Dunlop, M.J., (2023). Advances in linking single-cell bacterial stress response to population-level survival. *Curr Opin Biotechnol.* 79:102885. doi: 10.1016/j.copbio.2022.102885.

Bharath, L.P., Ip, B.C. & Nikolajczyk, B.S. (2017). Adaptive immunity and metabolic health: Harmony becomes dissonant in obesity and aging. *Comprehensive Physiology*, *7*(4), 1307-1337.

Bubnov, A.G., Buimova, S.A., Gushchin, A.A. & Izvekova, T.V. (2007). *Biotest Analysis - An Integral Method for Assessing the Quality of Environmental Objects.* Ivanovo: IGKhTU, 112. (In Russian).

Chiappin, S., Antonelli, G., Gatti, R. & De Palo, E.F. (2007). Saliva specimen: A new laboratory tool for diagnostic and basic investigation. *Clinica Chimica Acta*, *383*(1-2), 30-40.

Da Silva, F.G., Pola, N.M., Casarin, M., Silva, C.F.E. & Muniz, F.W.M.G. (2021). Association between clinical measures of gingival inflammation and obesity in adults: Systematic review and meta-analyses. *Clinical Oral Investigations, 25*(7), 4281-4298.

De Lemos, G.M., Resende, C.M.M., Campello, C.P., Ribeiro, I.S., Mendes, A.K., de Lima, E.L.S., ... & Muniz, M.T.C. (2024). Is oral microbiota associated with overweight and

obesity in children and adolescents? A systematic review. *Critical Reviews in Food Science and Nutrition*, *64*(13), 4275-4285.

Deo, P.N., Deshmukh, R. (2019). Oral microbiome: Unveiling the fundamentals. *Journal of Oral and Maxillofacial Pathology*, *23*(1), 122-128.

Dodge, Y., (2002). Statistical Data Analysis Based on the L1-Norm and Related Methods (Statistics for Industry and Technology). Birkhäuser / Springer. doi: https://doi.org/10.1007/978-3-0348-8201-9

Fleddermann, M., Knoll, A. & Koletzko, B. (2024). Safety and suitability of infant formula manufactured from extensively hydrolyzed whey protein compared to intact protein: A combined analysis of two randomized controlled studies. *Nutrients*, *16*(2), 245. https://doi.org/10.3390/nu16020245

Foratori-Junior, G.A., Guennec, A.L., Fidalgo, T.K.D.S., Cleaver, L., Buzalaf, M.A.R., Carpenter, G.H. & Sales-Peres, S.H.D.C. (2022). Metabolomic profiles associated with obesity and periodontitis during pregnancy: Cross-sectional study with proton nuclear magnetic resonance (1H-NMR)-based analysis. *Metabolites*, *12*(11), 1029.

Houten, J.,Van., (2023). A review for the special issue on *paramecium* as a modern model organism.*Microorganisms* 2023, *11*(4),937;https://doi.org/10.3390/microorganisms11040937

Humphrey, S.P., Williamson, R.T. (2001). A review of saliva: Normal composition, flow and function. *The Journal of Prosthetic Dentistry*, *85*(2), 162-169.

Khan, S., Barrington, G., Bettiol, S., Barnett, T. & Crocombe, L. (2018). Is overweight/obesity a risk factor for periodontitis in young adults and adolescents?: A systematic review. *Obesity Reviews*, *19*(6), 852-883.

Lamas, O., Marti, A. & Martinez, J. (2002). Obesity and immunocompetence. *European Journal of Clinical Nutrition*, *56*(3), S42-S45.

Landis, J. R., Koch, G. G., (1977). The measurement of observer agreement for categorical data**.** *Biometrics.* 1977 Mar;33(1):159-74.

Lavrov, V. I., Goncharov, I. B., Davydkin, A. F., Ivanov, A. P., Romanov, A. N., (1986). Paramecium test for toxic substances in the blood of humans exposed to conditions simulating weightlessness**.** *Kosm Biol Aviakosm Med.* 1986 Mar-Apr;20(2):58-60. [Article in Russian]

Mehdipour, A., Nattaj, M. H., Fateh, R., Aghaali, M., Qomeisi, R., Saleh, Ali., Neyestanaki, H., K., (2025). Association of salivary microbial, fungal population and salivary acidity with obesity in children. *J Dent (Shiraz).* 2025 Sep 1;26(3):210-219. doi:10.30476/dentjods.2024.102462.2364.

Neyraud, E., Palicki, O., Schwartz, C., Nicklaus, S. & Feron, G. (2012). Variability of human saliva composition: Possible relationships with fat perception and liking. *Archives of Oral Biology*, *57*(5), 556-566.

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., ... & Gakidou, E. (2014). Global, regional and national prevalence of overweight and obesity in children and adults during 1980-2013: A systematic analysis for the Global Burden of Disease Study 2013. *The Lancet*, *384*(9945), 766-781.

Pafomov, G., Burdyga, F. & Shirinova, M. (1980). Express method for determining the toxic properties of blood and lymph using paramecia in exo- and endotoxicoses. *Soviet Medicine*, 1, 42-45. (In Russian).

Pallaro, A., Barbeito, S., Taberner, P., Marino, P., Franchello, A., Strasnoy, I., ... & Slobodianik, N. (2002). Total salivary IgA, serum C3c and IgA in obese school children. *The Journal of Nutritional Biochemistry*, *13*(9), 539-542.

Piombino, P., Genovese, A., Esposito, S., Moio, L., Cutolo, P.P., Chambery, A., ... & Ercolini, D. (2014). Saliva from obese individuals suppresses the release of aroma compounds from wine. *PloS One*, *9*(1), e85611.

Rizzato, V.L., Lotto, M., Lourenço Neto, N., Oliveira, T.M. & Cruvinel, T. (2022). Digital surveillance: The interests in toothache-related information after the outbreak of COVID-19. *Oral Diseases*, 28, 2432-2441. https://doi.org/10.1111/odi.14012

Schamarek, I., Anders, L., Chakaroun, R.M., Kovacs, P. & Rohde-Zimmermann, K. (2023). The role of the oral microbiome in obesity and metabolic disease: Potential systemic implications and effects on taste perception. *Nutrition Journal*, *22*(1), 28.

Segal, A., Wong, D.T. (2008). Salivary diagnostics: Enhancing disease detection and making medicine better. *European Journal of Dental Education, 12*(Suppl 1), 22.

Syrjäläinen, S., Gursoy,U.K., Gursoy,M., Pussinen, P., Pietiäinen, M., Jula, A., Salomaa, V., Jousilahti, P., Könönen, E., (2019). Salivary Cytokine Biomarker Concentrations in Relation to Obesity and Periodontitis. *J Clin Med* . 2019 Dec 5;8(12):2152. doi: 10.3390/jcm8122152.

Tam, J., Hoffmann, T., Fischer, S., Bornstein, S., Gräßler, J. & Noack, B. (2018). Obesity alters composition and diversity of the oral microbiota in patients with type 2 diabetes mellitus independently of glycemic control. *PLoS One*, *13*(10), e0204724.

Thanakun, S., Pornprasertsuk-Damrongsri, S., Izumi, Y., (2017). Increased oral inflammation, leukocytes, and leptin, and lower adiponectin in overweight or obesity. *J Oral Diseases*. 17 May 2017. https://doi.org/10.1111/odi.12679

Toy, V., E., Ataoglu, T., Eltas, A., Otlu, H., G., Karabulut, A., B., (2023). Obesity as a modifying factor of periodontal therapy outcomes: local and systemic adipocytokines and oxidative stress markers**.** *Clin Oral Investig*. 2023 Jun;27(6):2763-2773. doi: 10.1007/s00784-022-04854-7.

Tremblay, M., Brisson, D. & Gaudet, D. (2012). Association between salivary pH and metabolic syndrome in women: A cross-sectional study. *BMC Oral Health*, *12*(1), 40.

Wu, Y., Chi, X., Zhang, Q., Chen, F., Deng, X., (2018). Characterization of the salivary microbiome in people with obesity. *PEERJ SECTION. Cardiovascular and Metabolic Disorders*. March 16, 2018. doi.10.7717/peerj.4458.

Zeng, M., Wang, X., Lan, Z., Guo, X., Jiang, Y., Wu, T., ... & Wang, C. (2022). Identification of new polymorphic positions in rDNA sequences of the intermediate fasciola forms. *Parasitology International*, 88, 102555. https://doi.org/10.1016/j.parint.2022.102555

Zeynalova, Zh. (2006). On the characteristics of dental anomalies in systemic diseases. *Proceedings of the 10th Congress of Russian Orthodontists*, 70. (In Russian).

Zeynalova, Zh. (2007). Histological characteristics of the tissue and gums in patients with systemic lupus erythematosus. *Health*, 4, 202-204. (In Azerbaijan).

Zeynalova, Zh. (2007). Morphological characteristics of the state of periodontal tissues in systemic lupus erythematosus. *Health*, 10, 198-201. (In Azerbaijan).