AI-Powered Novel BioAgeSense Device for Age Profiling Using Saliva

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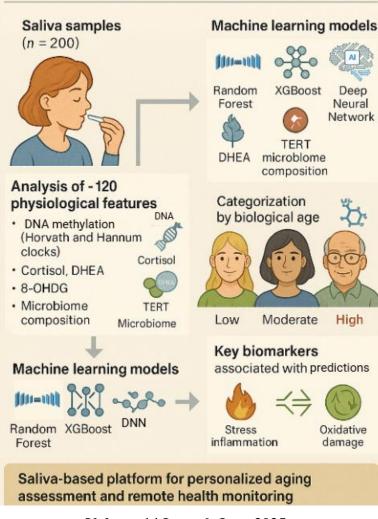
Abstract: This study introduces BioAgeSense, an AI-powered, non-invasive diagnostic device designed to estimate biological age using salivary biomarkers. Saliva samples from 200 participants (aged 20–70) were analyzed for physiological indicators such as DNA methylation (Horvath and Hannum clocks), cortisol, DHEA, 8-OHdG, TERT, inflammatory proteins, lactoferrin, urea, and microbiome composition. Biological age, derived from epigenetic clocks, served as the target variable. Machine learning models like Random Forest, XGBoost, and Deep Neural Networks (DNN) were developed, with the DNN achieving the best performance ($R^2 = 0.89$, MAE = 2.5, RMSE = 3.2). XGBoost and Random Forest followed closely, with R^2 values of 0.88 and 0.86, respectively. Participants were classified into low, moderate, and high-risk groups based on biological age acceleration scores. Key biomarkers related to stress, inflammation, and oxidative stress significantly influenced predictions. Distribution patterns of biomarkers included: cortisol (normal, ~5 ng/mL), DHEA (bell-shaped, ~2.5 ng/mL), 8-OHdG (right-skewed, ~10 ng/mL), Firmicutes/Bacteroidetes ratio (centered ~1.5), inflammatory proteins (~50 a. u.), lactoferrin (~4.5 µg/mL), and urea (~28 mg/dL). BioAgeSense shows strong potential as a scalable, saliva-based platform for personalized aging assessment and remote health monitoring in precision medicine.

BioAgeSense: Predicting Biological Age

from Salivary Biomarkers

Keywords: BioAgeSense, AI-driven, Saliva, Novel, Aging, Machine learning

Graphical Abstract

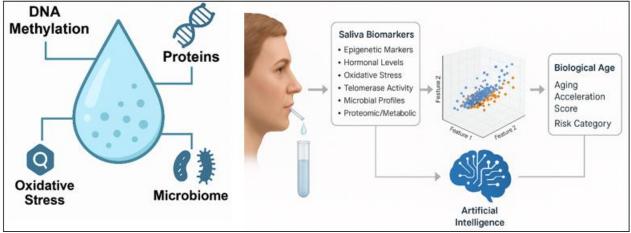


1. Introduction

Aging is a multifactorial biological process involving the gradual loss of cellular integrity, physiological resilience, and metabolic homeostasis (Selman & Pardo, 2021). While chronological age is a fixed measure based on the passage of time, biological age reflects the true physiological state of an individual, shaped by a complex interplay of genetics, environmental exposures, and lifestyle factors (Hartmann et al., 2023). Early identification of individuals experiencing accelerated biological aging is essential for guiding timely interventions to prolong healthspan and reduce the onset of chronic age-related diseases (Wallace et al., 2017). Traditional methods to assess biological aging have largely relied on invasive techniques such as blood sampling to measure telomere length, inflammatory cytokines, and DNA methylation status through high-throughput genomic assays (Li et al., 2024). Though highly informative, these approaches require specialized laboratory facilities, are cost-prohibitive for routine monitoring, and are often unsuitable for population-level screening (Vera & Blasco, 2012).

In contrast, BioAgeSense is a novel AI-driven salivary diagnostic platform which can predict aging via saliva, a promising alternative biofluid due to its ease of collection, non-invasive nature, and ability to reflect systemic physiological states. Saliva contains diverse biomolecules including DNA, RNA, proteins, hormones, and microbiotaderived metabolites (Ciurli et al., 2021) ---many of which correlate with aging processes and were integrated into the BioAgeSense system. In this study, BioAgeSense was deployed to analyze saliva samples from 200 participants aged 25 to 65 years. Biomarker selection within BioAgeSense was guided by established literature linking molecular changes to biological aging. Epigenetic markers such as DNA methylation patterns known from Horvath's and Hannum's clocks were included to infer cellular age (Baker & Sprott, 1988; Yusri et al., 2024). Hormonal biomarkers like cortisol and alpha-amylase were considered for their association with chronic stress, while oxidative stress markers such as 8hydroxy-2'-deoxyguanosine (8-OHdG) were measured as indicators of accumulated molecular damage (Palmer et al., 2019). Telomerase activity served as a proxy for replicative capacity, and shifts in oral microbiome composition provided insight into immune and metabolic alterations (Hau et al., 2015). Feature selection within BioAgeSense involved correlation filtering, entropy-based ranking, and recursive elimination to retain the most informative and non-redundant features for modeling.

To analyze this multi-dimensional dataset, BioAgeSense implemented three (Palmer et al., 2019) machine learning techniques: Random Forest, XGBoost, and Deep Neural Networks. Random Forest, an ensemble learning algorithm, constructed multiple decision trees to predict biological age by identifying biomarker splits that best reduced variance (Alzboon et al., 2025). This allowed BioAgeSense to identify key markers such as elevated cortisol and reduced telomerase activity, which frequently contributed to accelerated aging predictions (Shokhirev et al., 2024). XGBoost further enhanced model performance by iteratively minimizing prediction error while regularizing complexity crucial for biological datasets with many interdependent variables. It was particularly useful in uncovering non-linear relationships between oxidative and microbial biomarkers (Zhang et al., 2019). The Deep Neural Network (DNN) model in BioAgeSense was architected to capture high-dimensional biomarker interactions. The network translated raw biomarker inputs into abstract feature hierarchies through multiple hidden layers, allowing it to detect subtle epigenetic patterns and systemic changes related to aging (Galkin et al., 2021). Through backpropagation and fine-tuning, the DNN uncovered mechanistic signatures of aging that may not be apparent with classical models alone (Galkin et al., 2021). This integrative architecture empowered BioAgeSense to deliver accurate, personalized biological age predictions and risk stratification based on salivary biomarkers. Unlike traditional aging assessments that are often costly, invasive, and inaccessible at scale, BioAgeSense offers a practical, scalable, and non-invasive alternative suited for routine health monitoring, early detection of age-related decline, and individualized intervention planning.



Schematic Overview of the present investigation

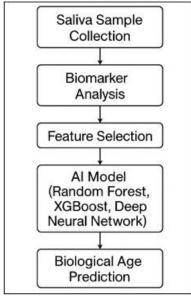
2. Materials and Methods

The study population consisted of 200 participants, ranging in age from 20 to 70 years, with an equal representation of male

and female subjects to ensure gender balance. Saliva samples were collected using a non-invasive, standardized method unstimulated saliva obtained through passive drool to maintain consistency and minimize variability in sample

composition. Participants were instructed to refrain from eating, drinking, or oral hygiene activities prior to sample collection to reduce external influences on salivary biomarkers. Individuals with chronic illnesses or those currently taking medications known to alter biomarker profiles were excluded from the study to avoid confounding effects and ensure the reliability and validity of the data collected. All participants provided written informed consent prior to participation. Confidentiality, anonymity, and the right to withdraw at any stage were fully ensured.

Flow Chart process



Biomarker Integration and Analysis

The primary target variable used within BioAgeSense was biological age, estimated using DNA methylation-based epigenetic clocks, specifically the Horvath and Hannum models. Salivary biomarkers were analyzed to assess agingrelated physiological changes. Cortisol, DHEA, alphaamylase, and 8-OHdG reflected stress and oxidative damage. TERT indicated telomerase activity, while the Firmicutes/Bacteroidetes ratio captured microbiome shifts. Inflammatory proteins, lactoferrin, and urea measured immune and metabolic status, supporting comprehensive biological age prediction through non-invasive sampling. The selected biomarkers served as input features for BioAgeSense, encompassing epigenetic, hormonal, oxidative stress, microbiome, and proteomic/metabolic categories.

Computational Framework for Biological Age Estimation

The core analytical pipeline of BioAgeSense began with structured preprocessing of quantitative biomarker data, followed by feature selection and scaling. Three machine learning models were integrated into the system: Random Forest (RF), XGBoost, and a custom Deep Neural Network (DNN). The DNN architecture included an input layer corresponding to selected biomarkers, multiple hidden layers with ReLU activation, dropout layers for regularization, and an output node predicting biological age. It was trained using the Adam optimizer and mean squared error as the loss function. RF and XGBoost models were optimized using GridSearchCV with 10-fold cross-validation on an 80% training subset. Performance evaluation was conducted on a 20% test set using R², MAE, and RMSE.

Additionally, biological age predictions were used to classify participants into risk groups (Low, Moderate, High), enabling the calculation of classification metrics such as Precision and Recall. This layered framework allowed BioAgeSense to combine interpretability, predictive accuracy, and biological insight for practical deployment in health monitoring. Each model's prediction output was subsequently integrated into the BioAgeSense risk stratification module to classify biological aging risk levels (Low, Moderate, High) based on the deviation between predicted biological and chronological age.

Training and Validation

The dataset was partitioned into training (80%) and testing (20%) subsets to evaluate the generalizability of predictions generated by BioAgeSense. To enhance robustness and prevent overfitting, the platform implemented 10-fold crossvalidation on the training data. Hyperparameter tuning for classical machine learning models like Random Forest and XGBoost was conducted within BioAgeSense using GridSearchCV, enabling systematic optimization of model parameters for improved performance. For the Deep Neural Network (DNN), the Adam optimizer was employed to minimize the mean squared error (MSE) loss function. The DNN training module in BioAgeSense included dropout layers and regularization techniques to ensure stability and reliability when processing high-dimensional salivary biomarker inputs, ultimately supporting accurate and scalable biological age prediction.

3. Results

The predictive performance of the models within BioAgeSense was evaluated using standard regression metrics: the coefficient of determination (R²), mean absolute error (MAE), and root mean squared error (RMSE). Among the three algorithms tested, the Deep Neural Network (DNN) exhibited the highest predictive accuracy, achieving an R² score of 0.89, an MAE of 2.5, and an RMSE of 3.2 indicating strong reliability in estimating biological age from salivary biomarkers. XGBoost performed comparably, with an R² of 0.88, MAE of 2.9, and RMSE of 3.7. The Random Forest model also demonstrated robust performance, with an R² of 0.86, MAE of 3.2, and RMSE of 4.1. These results confirm that all three models embedded in BioAgeSense effectively capture the underlying biological signals of aging, with the DNN providing the most precise and generalizable predictions.

Distribution of Biological Aging Acceleration

Sample outputs generated by the BioAgeSense predictive model reveal distinct inter-individual variations between chronological and biological age, underscoring the system's sensitivity to subtle physiological differences (Table 1). For example, Participant BIO-00412, chronologically aged 45.0 years, exhibited a predicted biological age of 52.4 years, yielding an acceleration score of +7.4 years and a classification in the High-Risk category. In contrast, Participant BIO-00235 (age 39.0) demonstrated decelerated aging with a biological age of 36.7 years (-2.3 years), falling into the Low-Risk group. Participant BIO-00117 (age 58.0) showed a mild acceleration of +2.1 years and was categorized as Moderate Risk, while BIO-00789 (age 62.0) experienced a pronounced acceleration of +8.5 years, aligning with the High-Risk group. Participants BIO-00308 (age 33.0) and BIO-00294 (age 29.0) both exhibited biological age reductions of -1.8 and -0.5 years, respectively, and were classified as Low Risk. Other cases such as BIO-00651 (age 50.0, +3.6 years) and BIO-00566 (age 55.0, +2.3 years) fell within the Moderate Risk category. Notably, Participant BIO-00804 (age 51.0) had a biological age of 58.9 years (+7.9), indicating accelerated aging, whereas BIO-00921 (age 47.0) showed a deceleration of -1.9 years, emphasizing the model's capacity to detect nuanced differences in aging trajectories across individuals.



Figure 1: Developed prototype BioAgeSense

Table 1: Sample Output with Predicted Biological Age and
Risk Categories

Participant ID	Chronological Age (yrs)	Predicted Biological	Acceleration Score (yrs)	Risk Category
BIO-00412	45.0	Age (yrs) 52.4	+7.4	High
BIO-00235	39.0	36.7	-2.3	Low
BIO-00117	58.0	60.1	+2.1	Moderate
BIO-00789	62.0	70.5	+8.5	High
BIO-00308	33.0	31.2	-1.8	Low
BIO-00651	50.0	53.6	+3.6	Moderate
BIO-00804	51.0	58.9	+7.9	High
BIO-00294	29.0	28.5	-0.5	Low
BIO-00566	55.0	57.3	+2.3	Moderate
BIO-00921	47.0	45.1	-1.9	Low

The distribution of biological aging risk among the 200 study participants was assessed using a pie chart generated by BioAgeSense, based on model-derived biological age acceleration scores. The analysis revealed that approximately 35% of individuals were classified as Low Risk, indicating decelerated or age-appropriate biological aging. Another 40% fell into the Moderate Risk group, representing mild to moderate acceleration of biological age. The remaining 25% were categorized as High Risk, suggesting marked advancement in biological aging relative to chronological age. This stratification underscores the heterogeneity of aging trajectories within the population and demonstrates the potential of saliva-based biomarker profiling via BioAgeSense for personalized, non-invasive risk assessment.

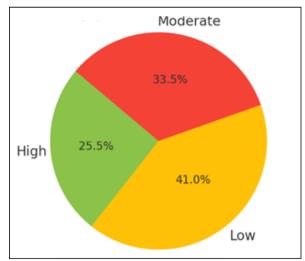


Figure 2: Biomarker distribution

Cortisol (ng/mL)

The histogram (Figure 3) for salivary cortisol levels, as analyzed by BioAgeSense, reveals a nearly normal distribution centered around 5 ng/mL, aligning with established physiological norms for baseline diurnal secretion in healthy adults. A mild right skewness is observed, suggesting a subset of individuals with elevated cortisol levels, potentially indicative of chronic psychological or physiological stress. As cortisol is a principal output of the hypothalamic-pituitary-adrenal (HPA) axis, such elevations may reflect increased allostatic load a recognized driver of accelerated biological aging. This distribution pattern highlights cortisol's diagnostic relevance in detecting stressrelated dysregulation and supports its integration into the BioAgeSense platform as a key biomarker for age-associated physiological decline.

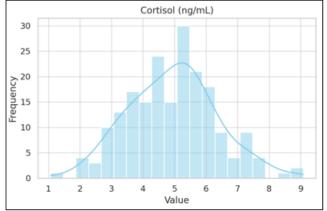


Figure 3: Salivary cortisol levels, as analyzed by BioAgeSense

DHEA (ng/mL)

The distribution of dehydroepiandrosterone (DHEA) concentrations, as assessed by BioAgeSense, exhibits a bell-shaped curve peaking around 2.5 ng/mL typical for mid-life adults (Figure 4). The histogram reflects generally balanced adrenal androgen output across the cohort, with a few low-end outliers. Given DHEA's inverse relationship with chronological age and its critical role in regulating immune function, mood, and energy metabolism, decreased levels serve as indicators of endocrine aging. These findings contribute to higher biological age predictions within the BioAgeSense framework. The presence of individuals with markedly reduced DHEA reveals its value as a predictive biomarker for early identification of accelerated aging.

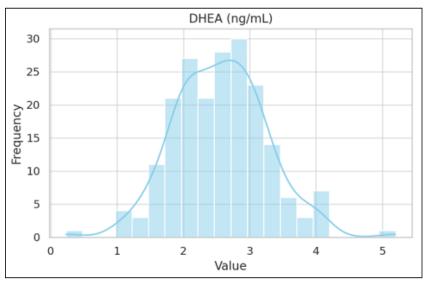


Figure 4: DHEA assessement by BioAgeSense

Alpha-Amylase (U/mL)

Salivary alpha-amylase levels, as profiled by BioAgeSense, display a positively skewed histogram with a concentration of values in the lower range and a trailing tail of elevated readings (Figure 5). This enzyme, released in response to sympathetic nervous system activation, functions as a sensitive marker of acute stress. The observed skewness reflects rapid, transient secretory responses to psychological or physical stimuli. While most participants fall within normative limits, elevated levels in certain individuals suggest episodic stress responses that may contribute to biological age acceleration. These findings support the inclusion of alpha-amylase within BioAgeSense as a relevant biomarker for capturing stress-related physiological variability.

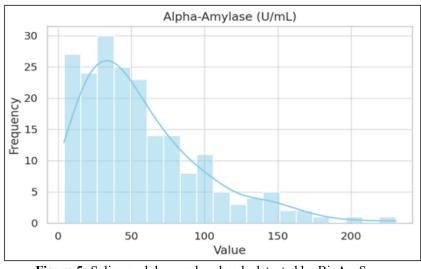


Figure 5: Salivary alpha-amylase levels detected by BioAgeSense

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8-OHdG (ng/mL)

The histogram of 8-hydroxy-2'-deoxyguanosine (8-OHdG) concentrations, as analyzed by BioAgeSense, reveals a moderate right-skew, indicating varying levels of oxidative DNA damage across the cohort. The central tendency is near 10 ng/mL, with outliers exceeding 16 ng/mL (Figure 6). As a validated biomarker of oxidative stress and genomic

instability, both central to aging 8-OHdG which reflects cumulative exposure to reactive oxygen species (ROS) and diminished antioxidant defenses. Participants in the upper quartile exhibit signatures of increased cellular damage, reinforcing 8-OHdG's value within the BioAgeSense platform for identifying individuals at risk of accelerated biological aging due to oxidative stress.

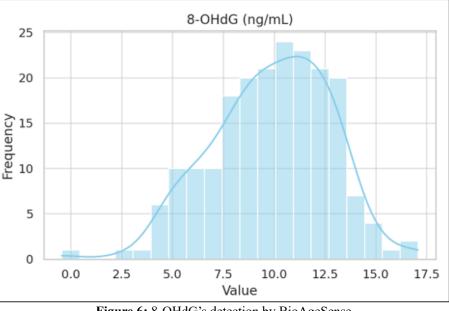


Figure 6: 8-OHdG's detection by BioAgeSense

TERT (Relative Units)

Telomerase reverse transcriptase (TERT) levels, as profiled by BioAgeSense, are narrowly distributed around a mean of 1.0 relative units, indicating relatively consistent telomerase activity across the study population (Figure 7). The histogram shows a mild left skew, with a subset of participants exhibiting reduced TERT expression potentially signaling impaired telomere maintenance capacity. Low telomerase activity is closely linked to cellular senescence and reduced regenerative potential, both of which are key hallmarks of aging. These findings support the integration of TERT within the BioAgeSense framework as a critical molecular marker capable of differentiating between normal and accelerated aging trajectories, particularly when evaluated alongside DNA methylation age estimates.

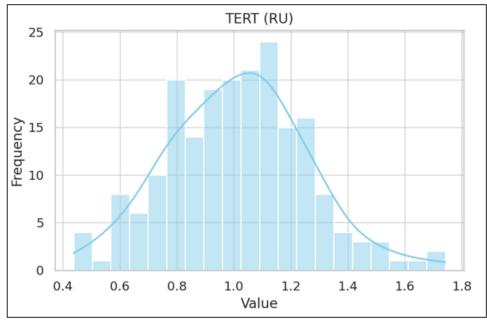


Figure 7: TERT analysis by BioAgeSense

Firmicutes/Bacteroidetes Ratio (F/B Ratio)

The histogram for the Firmicutes/Bacteroidetes (F/B) ratio analyzed through BioAgeSense as a proxy for oral microbiota balance shows a broad, symmetrical distribution centered around 1.5 (Figure 8). This ratio plays a key role in metabolic regulation, systemic inflammation, and gut–brain axis signaling, all of which are known to affect the aging process. Deviations from this central value, whether elevated or suppressed, may indicate oral dysbiosis, dietary imbalances, or underlying systemic conditions. Individuals with abnormal ratios are potentially at greater risk for inflammatory aging and metabolic dysfunction, supporting the integration of microbiome-derived metrics within the BioAgeSense framework for comprehensive biological age prediction.

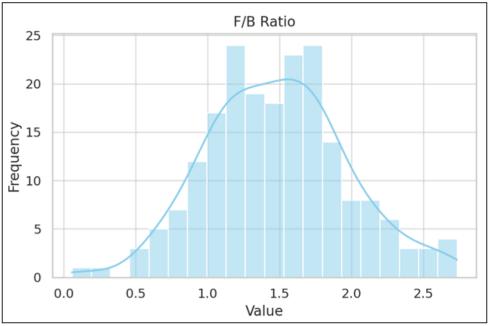


Figure 8: Firmicutes/Bacteroidetes (F/B) ratio analyzed through BioAgeSense

Inflammatory Proteins (Arbitrary Units)

Inflammatory protein levels in saliva, as measured by BioAgeSense, exhibit a wide distribution with a peak around 50 arbitrary units (a. u.), aligning with patterns of chronic low-grade inflammation ("inflammaging") commonly seen in middle-aged and older adults (Figure 9). The histogram reveals considerable variability, likely reflecting heterogeneous immune statuses across the cohort. Individuals in the upper percentiles may be experiencing persistent systemic or localized (oral) inflammation both of which are strongly associated with accelerated biological aging. This distribution highlights the importance of inflammatory profiling within the BioAgeSense framework, reinforcing its utility in stratifying aging risk based on immune system dysregulation.

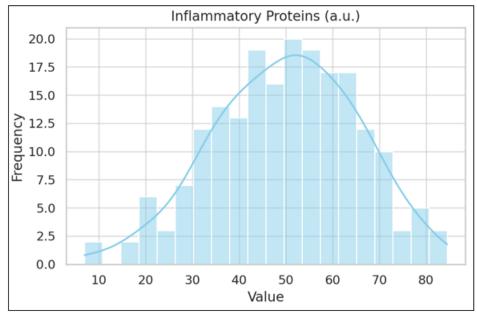


Figure 9: Inflammatory protein levels in saliva, as measured by BioAgeSense

Lactoferrin (µg/mL)

The histogram of lactoferrin levels, as assessed by BioAgeSense, reveals a normal distribution with a central peak at $4.5 \,\mu$ g/mL. Lactoferrin, an iron-binding glycoprotein, is integral to mucosal immunity and antimicrobial defense. Salivary concentrations vary with age, immune function, and oral microbial dynamics (Figure 10). The observed symmetry

and narrow spread suggest homeostatic regulation in most participants, while lower outliers may reflect weakened mucosal immunity. Given lactoferrin's dual relevance to immune and microbiome-related aging, its inclusion in BioAgeSense provides meaningful insight into mucosal immune health, supporting its role in non-invasive monitoring of immunosenescence and aging trajectories.

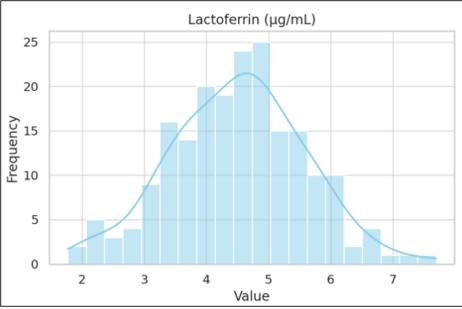


Figure 10: Lactoferrin levels, as assessed by BioAgeSense

Urea (mg/dL)

Salivary urea levels, as analyzed by BioAgeSense, display a near-Gaussian histogram centered around 28 mg/dL (Figure 11). As a byproduct of protein metabolism, urea serves as a reliable metabolic marker influenced by renal function, protein intake, and hydration status. The histogram's symmetry suggests metabolic stability in most participants, while higher-end outliers may indicate catabolic stress or early metabolic dysregulation. Given its role in systemic homeostasis, urea contributes significantly to the BioAgeSense multi-biomarker framework for predicting biological age, particularly when interpreted alongside oxidative stress and inflammatory profiles.

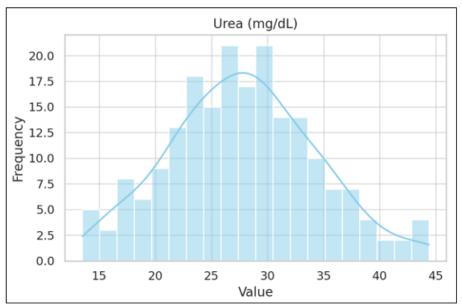


Figure 11: Salivary urea levels, as analyzed by BioAgeSense

4. Discussion

This study demonstrates the feasibility and effectiveness of using salivary biomarkers integrated with artificial intelligence through the BioAgeSense platform to estimate biological age and stratify aging-related risk. By combining multi-dimensional data-including hormonal, oxidative, epigenetic, microbiome, and immune-metabolic markers-BioAgeSense provides a holistic view of systemic aging via a non-invasive and easily accessible medium. The strong performance of all three predictive models, particularly the Deep Neural Network ($R^2 = 0.89$), highlights the platform's capability to deliver accurate, personalized age assessments (Ying et al., 2024). The observed variations between predicted biological and chronological age expressed as the acceleration score-enabled identification of individuals experiencing early biological aging, potentially driven by stress, inflammation, or metabolic dysregulation. Elevated cortisol and 8-OHdG levels, for instance, were frequently linked with accelerated aging within the BioAgeSense system, supporting their roles in allostatic load and oxidative stress (Polsky et al., 2022). Similarly, reduced DHEA and TERT levels reflected endocrine and cellular senescence. These insights reinforce the clinical utility of salivary biomarkers, processed through BioAgeSense, as early indicators of physiological decline (Maciejczyk et al., 2022; Polsky et al., 2022). The use of machine learning within the platform not only facilitated accurate prediction but also enabled efficient integration of complex, nonlinear relationships among diverse biomarkers. Furthermore, the risk stratification feature (Low, Moderate, High) built into BioAgeSense offers actionable outputs for targeted intervention and lifestyle adjustment. With its non-invasive sampling and potential for remote deployment, BioAgeSense presents a scalable solution for population-level screening and preventive health monitoring. Nonetheless, limitations exist. The cross-sectional design limits causal inference, and external validation in independent cohorts is necessary to generalize findings. Future integration of longitudinal data and lifestyle factors could further enhance the precision, interpretability, and clinical relevance of BioAgeSense.

5. Conclusion

This study successfully demonstrates the potential of BioAgeSense, an AI-powered platform, to integrate salivary biomarkers with advanced machine learning models for non-invasive estimation of biological age and stratification of aging-related health risks. The high predictive accuracy—particularly from the Deep Neural Network—emphasizes the strength of combining multidimensional biomarker data with artificial intelligence for personalized aging assessments. The use of saliva enhances accessibility, compliance, and scalability, positioning BioAgeSense as a viable tool for population-level screening and remote health monitoring.

Despite these promising outcomes, several challenges remain. The cross-sectional design limits causal inference and precludes monitoring temporal changes in biological age. Additionally, external lifestyle factors such as diet, sleep, and physical activity were not captured, yet they significantly influence biomarker expression and aging dynamics. Model interpretability, especially in deep learning, remains a key limitation for clinical translation without explainable AI frameworks. Furthermore, the current model requires external validation in larger, ethnically diverse cohorts to ensure robustness and generalizability.

Looking forward, future research should prioritize longitudinal studies to track changes in biological age over time and in response to interventions. Enhancing BioAgeSense with multi-omics integration (e.g., transcriptomics, metabolomics) and real-time data from wearable sensors may further strengthen predictive accuracy and biological relevance. The development of transparent, interpretable AI and user-friendly risk communication tools will be essential for clinical and public health adoption. Ultimately, BioAgeSense represents a foundational step toward scalable, non-invasive biological age monitoring with the potential to transform personalized preventive medicine and healthy aging strategies.

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