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CORTISOL HORMONE AND STRESS LEVELS: COLORIMETRIC ASSESSMENT WITH ARTIFICIAL INTELLIGENCE SUPPORTED IMAGE PROCESSING METHOD

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Highlights

- Computer Vision
- Convolutional neural networks
- Cortisol hormone
- Occupational stress
- Psychosocial risk assessment

Graphical Abstract

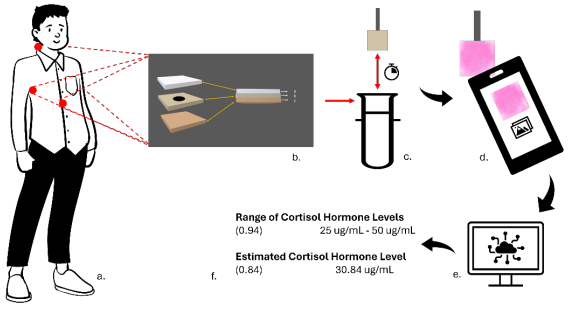

Figure Operating principle of the proposed system

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ABSTRACT: This study presents a new method for determining cortisol hormone levels, a key biomarker of stress, using microfluidic pads to collect sweat samples. The pads facilitate the colorimetric detection of cortisol levels via the blue tetrazolium method. The resulting color change is analytically assessed using Convolutional Neural Networks (CNN), Decision Trees, and Vector Regression, alongside advanced image processing techniques. The developed algorithm is robust, providing reliable results despite hardware variations and color distortion, enhancing the system's applicability and generalizability across different environments. Validation studies conducted with ELISA and a colorimeter revealed that the system achieved an accuracy of 84.2% in determining users' cortisol levels. Additionally, psychosocial stress levels were assessed using the Copenhagen Psychosocial Risk Assessment and the Perceived Stress Scale tests during the collection of sweat samples from 20 participants. The results demonstrated a significant correlation between cortisol levels and stress, confirming the method's reliability and effectiveness in various applications.

Keywords: *Computer vision, Convolutional neural networks, Cortisol hormone, Occupational stress, Psychosocial risk assessment*

1. INTRODUCTION

This study introduces a novel method for determining cortisol hormone levels, a key biomarker of stress, which holds particular importance in occupational health and safety. Elevated stress levels in the workplace can lead to increased accidents, diminished job performance, and long-term health consequences (1). Physically, stress is linked to conditions such as heart disease and hypertension, while mentally, it can lead to anxiety, depression, and burnout, negatively impacting both personal well-being and workplace productivity (2).

Traditional stress measurement methods, such as self-report surveys, are subjective and may lack accuracy due to their reliance on participants' perceptions (3). In contrast, cortisol, a physiological biomarker produced in response to stress, offers an objective means of assessing stress levels (3). While Enzyme-Linked Immunosorbent Assay (ELISA) is considered the gold standard for cortisol measurement, it is costly, time-consuming, and requires specialized personnel, limiting its feasibility in large-scale or rapid-response applications (4).

Psychosocial stress in humans activates the hypothalamic-pituitary-adrenal (HPA) axis, triggering the release of key hormones such as corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and most notably, cortisol (33). This hormonal cascade plays a critical role in the body's effort to

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restore homeostasis through complex physiological and psychological adaptations (34,35). Cortisol, secreted by the adrenal glands, facilitates energy mobilization, modulates metabolism, and regulates immune responses during stressful conditions. However, chronically elevated cortisol levels are associated with a range of adverse health effects, including metabolic disorders, immune suppression, and cardiovascular risks (36).

The concept of *allostasis*, introduced by McEwen, Sterling, and Eyer, explains the biological cost of stress exposure. Persistent activation of the HPA axis and prolonged exposure to stress mediators such as cortisol, catecholamines, and cytokines can lead to systemic dysregulation and tissue damage (37,38). Because cortisol displays a circadian rhythm, with peak secretion within the first hour of awakening, timing of sampling is critical for accurate assessments (38).

Numerous analytical techniques have been used for cortisol detection, including ELISA, mass spectrometry, chemiluminescent immunoassays (CLIA), lateral flow assays (LFIA), and electrochemical biosensors (39,40). Each offers trade-offs in terms of sensitivity, cost, processing time, and point-of-care applicability (41). Sweat has emerged as a promising matrix for non-invasive cortisol monitoring, especially through wearable microfluidic devices that offer continuous and real-time stress tracking (42). The blue tetrazolium method provides a visually interpretable and low-cost means of quantifying cortisol via color change (43).

Building on this foundation, this study developed a rapid and cost-effective cortisol detection platform utilizing microfluidic sweat patches and colorimetric measurement with blue tetrazolium. RGB values from the color reaction were extracted via image processing and analyzed using machine learning algorithms including Convolutional Neural Networks (CNN), Decision Trees, and Vector Regression (5–7). In prior work, RGB-based quantification using ESP32 camera modules showed correlation with cortisol concentration as low as 0.8 ng/mL, supporting the platform's diagnostic potential (44).

Beyond laboratory analysis, recent studies have explored the integration of machine learning and wearable sensor technologies to monitor cortisol-associated stress in real-time. For example, a system combining salivary cortisol with wearable physiological sensors (e.g., heart rate, skin temperature, activity) was used to evaluate stress in elderly adults (45). Using data collected during a Trier Social Stress Test (TSST), features from electrodermal activity (EDA), blood volume pulse (BVP), interbeat interval (IBI), and skin temperature (ST) were extracted and analyzed. Classification using logistic regression, random forest, k-NN, support vector machines, and LSTM neural networks revealed that logistic regression achieved the highest micro-averaged F1 and AUC scores (0.95 and 0.81, respectively), while LSTM models improved AUC by 11% (45,46).

Nath et al. designed a smart wristband system that leveraged salivary cortisol as the biological reference while continuously collecting EDA, BVP, and ST signals. In a year-long study involving 40 older adults, the combined use of these physiological features enhanced classification accuracy (F1-score = 0.92, accuracy = 94%) between stressed and non-stressed states (46). A voice-assisted prototype was also developed to provide real-time feedback, highlighting the potential of such systems for personalized stress management in aging populations.

Historically, subjective stress measurement methods such as the DASS-21 and STAI have faced criticism for low objectivity and inconsistency (47,48). Cortisol-based systems offer a more biologically grounded approach, yet studies integrating cortisol with consumer-grade electronics for stress monitoring remain limited. Charness et al. highlighted that older adults show higher acceptance for wrist-based devices and adapt more quickly to their use, suggesting that wearable cortisol-based stress monitors may be especially effective in this demographic (49).

Together, these findings underscore the need for affordable, non-invasive, and objective stress detection systems that combine validated biomarkers like cortisol with machine learning analytics. The present study addresses this need by proposing a novel, low-cost solution that integrates sweat-based cortisol detection with automated image processing and AI-based interpretation. This integrated platform

holds potential for broad application in occupational health surveillance, elderly care, and consumer wellness monitoring.

2. MATERIAL AND METHODS

In this study, the following materials were utilized: hydrocortisone acetate (Sigma 5003-3), tetramethylammonium hydroxide (Sigma 7559-2), ethanol (99% ACS grade), methanol (99% ACS grade), blue tetrazolium (Sigma 1871223), CHR 1 Whatman chromatography paper, Elabscience Human Cortisol Immunosorbent Assay Kit (E-EL-0157), Bio Rad PR 4100 Absorbance Microplate Reader, and 3nh CR1 Portable Smart Color Reader Colorimeter.

This study involves several key steps: chemical method development, software system creation, field studies, and validation. The chemical method focuses on selecting and applying appropriate techniques for cortisol analysis, prioritizing sensitivity, accuracy, and repeatability. Software system development includes designing and implementing tools for data analysis, modeling, and simulation, integrating programming languages and analytical tools.

Field studies include collecting and analyzing real-world data, combining chemical analysis and psychosocial assessments to test the system in practical settings. In this study, sweat collection pads were placed in the axillary (armpit) region for all participants in order to ensure standardization of sampling. This anatomical region was preferred because it has a high density of eccrine sweat glands and is more protected against environmental factors. Thus, it was aimed to minimize physiological and environmental variability.

The study was conducted with a homogeneous group of male industrial workers between the ages of 30 and 40. Exclusion criteria were expanded to eliminate potential confounders that could affect cortisol levels. Individuals who received any corticosteroid treatment (oral, topical or inhaled) in the last six months and those with chronic conditions requiring steroid use such as asthma, COPD and autoimmune diseases were not included in the study. In addition, individuals with metabolic disorders such as hypertension and diabetes were also excluded.

The validation studies conducted within this scope included both laboratory tests and field applications. ELISA and colorimetric methods were evaluated comparatively; the accuracy and reliability of the system were demonstrated with statistical analyses. Additionally, by measuring factors such as stress, workload and workplace conditions through psychosocial risk assessments, strategic information was obtained to improve worker health and productivity.

2.1. Chemical Method Studies

2.1.1. Preparation of cortisol stock solution

Cortisol stock solutions were freshly prepared weekly using hydrocortisone acetate and ethanol.

2.1.2. Chemical color reaction method

A 5% w/w tetramethylammonium hydroxide solution was prepared by diluting 5 mL of an aqueous solution in 45 mL of methanol. A second solution containing 100 mg of blue tetrazolium dissolved in 50 mL of methanol was also prepared. Both solutions were mixed in equal amounts and stored at room temperature.

2.1.3. Microfluidic design

The microfluidic system design consists of three layers in 1.5 cm^2 squares. The top layer is a sweat-absorbing cellulose-based pad, the middle layer channels sweat to the analysis layer using natural resin for point transitions, and the bottom layer is the analysis layer made of chromatography paper. The system collects sweat samples, which are then exposed to a reactive solution, causing a color change in the

analysis region. CHR 1 Whatman chromatography paper, cellulose-based pads, and resin were used in the design.

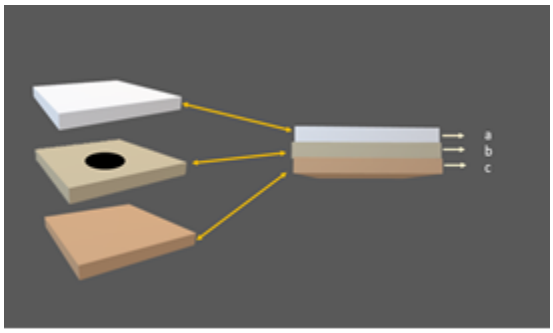

Figure 2.1. Design Schematic of the System (a) Analysis Layer (b) Intermediate Channel Layer (c) Sweat Absorption Layer

2.1.4. Work with Cortisol Standards

The obtained standards were tested on the developed system. Cortisol standards were applied to the sweat absorption layer, and after the transfer to the analysis layer, the system was immersed in a solution containing blue tetrazolium and buffer solutions to induce color change.

2.2. Software Development

Colorimetric detection methods are particularly prominent in biotechnological applications due to their practical use, yielding rapid results, high accuracy, and low-cost production and usability.

The Python programming language was used for software development. For image processing, the OpenCV, scikit-image, and Pillow libraries were employed; for dataset creation, Pandas and Numpy libraries were used. TensorFlow, Keras, and PyTorch libraries were utilized for Convolutional Neural Networks, and Scikit-Learn and Statsmodels libraries were chosen for Regression Analysis.

The full software architecture of the proposed system is presented in Figure 2.2, encompassing the preprocessing, CNN-based feature extraction, and regression-based quantification stages for sweat cortisol analysis.

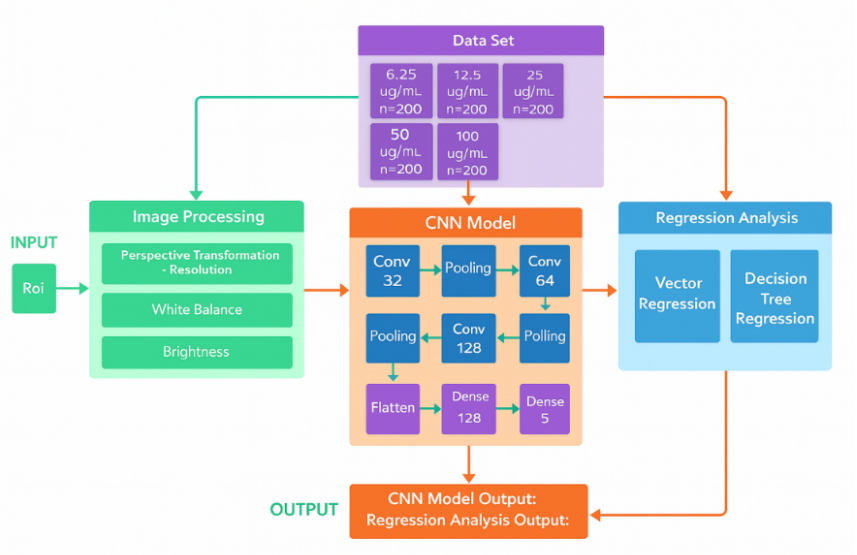

Figure 2.2. Artificial intelligence-supported colorimetric analysis system software architecture.

This architecture includes three basic components, namely image processing, feature extraction, and regression analysis. The images taken as input are first normalized with operations such as white consistency, part correction, and perspective view. The processed images are analyzed with the Convolutional Neural Network (CNN) model in a way that corresponds to data settings representing different cortisol distributions. Within the CNN architecture, meaningful features are highlighted from the image with multi-layered evolution, pooling, dense connection, and smoothing operations. These obtained features are evaluated with vector regression and decision tree regression models to estimate the cortisol point. The system provides an end-to-end solution for low redundancy and fast biomarker analysis.

2.2.1. Image preparation for CNNN-based model

In this study, 200 images were captured for each reference concentration (6.25, 12.5, 25, and 50 ppm), using three different smartphone cameras in four distinct environmental conditions, yielding a total of 1,000 training frames. To address the risk of overfitting associated with the limited dataset (n=200 per class), a 5-fold cross-validation approach was implemented during model training. In this method, each subset served once as the validation set while the remaining subsets were used for training, ensuring the model's generalizability and robustness across varying image distributions.

To standardize image acquisition and reduce variability from device-specific processing, the "Open Camera" application was used instead of native camera apps. Moreover, due to heterogeneous lighting and color calibration across devices and settings, image preprocessing techniques were applied—including brightness, white balance, and contrast adjustments—to improve the quality and consistency of the input data. These preprocessing steps were essential to optimize feature extraction and facilitate more accurate learning in the convolutional neural network (CNN) pipeline.

2.2.2. Image Processing

Standardizing image dimensions is essential for machine learning models, which require fixed-size inputs. Captured images were resized to 600x600 pixels, and the color change regions were isolated using perspective transformation, with all regions resized to 100x100 pixels. This process corrected distortions caused by camera angles using `cv2.getPerspectiveTransform` and `cv2.warpPerspective`. Brightness adjustments were made by converting the images to HSV and Lab color spaces, correcting brightness through the Lightness (L) channel for more uniform adjustments while preserving color accuracy.

Consequently, the brightness values of all images used were standardized using the following mathematical functions [8,9]:

The brightness of all images was standardized by converting them to the LAB color space, where the L channel (luminance) represents pixel brightness. The average brightness of the L channel was calculated to adjust the overall brightness of the image. (2.1).

$$L = \sum_{Ni=1} Li \qquad (2.1)$$

The target brightness value is determined as the average of the brightness values of 1,000 images taken with three different optical devices in different environments. The difference between the target brightness value and the average brightness value is used to calculate a correction factor (3.2).

$$\Delta L = Target\ Brightness\ Value - L^{-} \qquad (2.2)$$

This factor is applied to adjust the brightness of the image to the target brightness. The brightness correction factor is applied to the brightness value of each pixel in the L channel, bringing the brightness value closer to the target value (3.3).

$$k = Target\ Brightness\ Value - L \qquad (2.3)$$

The corrected brightness value is calculated as the product of the original brightness value and the correction factor for each pixel (3.4).

$$L_{corrected} = k * L_{original} \qquad (2.4.)$$

The corrected L channel is combined with the a and b channels and converted back to BGR to adjust overall brightness, ensuring consistency. Additionally, white balance is equalized to correct color variations from different lighting conditions, ensuring accurate and uniform color representation across all images.

To adjust white balance, gray reference points are used to ensure that red, green, and blue components are equal. Variations in lighting can cause imbalances in these components. The LAB color space, which represents colors closer to human perception, is used for white balance correction by converting the image to LAB and applying adjustments (10,11).

The gray reference is calculated (2.5):

$$Gray\ Reference = \frac{1}{N \sum_{Ni=1} Li} \qquad (2.5)$$

The image is converted to the LAB color space, and the averages of the a (green-red) and b (blue-yellow) components are calculated. The difference from the reference point (128) is used to adjust these components, ensuring accurate white balance. The image is then converted back to RGB. These preprocessing steps enhance image quality, improving the accuracy and performance of the machine learning model. Careful selection of these steps is essential for reliable results.

2.2.3. Concentration determination using Convolutional Neural Networks (CNN)

The Convolutional Neural Network (CNN) model, implemented using TensorFlow and Keras, was designed for image processing and classification. It includes three convolutional layers: Conv 32, Conv 64, and Conv 128, which progressively extract more complex features from the input image. Pooling layers, using max-pooling, reduce feature map dimensions, lowering computational costs and improving generalization. The output of the convolutional and pooling layers is flattened into a vector, which is fed into two fully connected layers: Dense 128 for learning abstract features and Dense 5 for final predictions. ReLU activation is used for efficiency, while a linear activation function is applied in the output layer for regression analysis (12,13,14).

2.2.4. Regression prediction model

Vector regression models multiple dependent variables simultaneously, providing flexibility in predicting interconnected systems. It is often associated with support vector machines (SVM) and works by finding a hyperplane that separates data points for prediction (15). The basic formula is;

$$f(x) = w_t + b \qquad (2.6)$$

where w is the weight vector, x is the input vector, and b is the bias term. The objective function, which includes error tolerance, is expressed as follows:

$$\frac{1}{2} \|w\| 2 + C \sum_{Ni=1} \varepsilon_i \qquad (2.7)$$

where C is the penalty parameter for error, and i are the error terms.

Decision tree regression predicts continuous variables by dividing data based on decision rules, splitting the data into segments and predicting values by reaching the tree's leaf nodes. Information gain,

calculated through entropy, guides the splitting process. Decision trees are favored for their interpretability and ability to handle both qualitative and quantitative data without special preprocessing. In this study, vector regression and decision tree regression were used together to predict cortisol hormone levels. Vector regression handled multiple stressors and health indicators, while decision tree regression helped identify patterns in the dataset, enhancing model interpretability and accuracy.

Root Mean Square Error (RMSE) and Mean Absolute Error (MAE) are error measures, and lower values indicate higher performance [10,11]. For example, RMSE equals zero when a model performs well. The error in predicting a time series observed at time t is expressed by the formula e_t in Equation 2, where r_t represents the observed time series and p_t represents the predicted time series [16].

$$e_t = r_t - p_t \qquad (2.9)$$

The error metrics and their formulas are as follows:

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(e_i)^2}$$

$$MAE = \frac{1}{n}\sum_{i=1}^{n}|e_i| \qquad (2.10)$$

The success rate indicates how much of the absolute errors between the predicted and actual values are acceptable compared to the average of the actual values. Its formula is:

$$Success\ Rate = \left(1 - \frac{MAE}{Average\ of\ Actual\ Values}\right) * 100$$
$$Success\ Rate = \left(1 - \frac{RMSE}{Average\ of\ Actual\ Values}\right) * 100 \qquad (2.11)$$

MAE (Mean Absolute Error) measures the average absolute differences between actual and predicted values, while the success rate reflects how small this error is relative to the mean of actual values, indicating model accuracy. In Table 4.13, success rates and performance metrics, such as MAE, RMSE, and standard deviation, varied when predicting cortisol levels from ELISA measurements. Lower MAE, RMSE, and standard deviation suggest more accurate and consistent predictions, with higher success rates indicating better model performance (16,17).

2.3. Validation (comparison with ELISA and colorimetry)

2.3.1. Participant selection and sampling procedure

The study involved 32 employees at a foundry in Gedik Holding, Sakarya, Turkey. Sweat-absorbing pads were attached to participants' armpits and backs for 6 hours to collect samples for cortisol analysis using microfluidic layers, ELISA, and colorimetric methods. After excluding participants who didn't complete the survey (n=5), lost pads (n=3), or had unusable pads (n=4), samples from 20 participants were evaluated. Individuals with hypertension, diabetes, or obesity were excluded due to their potential impact on cortisol levels. The samples were then transported to the lab under a cold chain.

2.3.2. Reference measurement with ELISA protocol

Sweat-absorbing pads worn for 6 hours were soaked in 1 ml phosphate buffer (pH 7) and incubated for 15 minutes. After centrifuging at 4500 rpm for 15 minutes, the liquid portion was transferred to sterile tubes for analysis. ELISA studies were conducted using the Elabscience Human Cortisol Immunoassay kit, and a portion of the sweat samples was analyzed colorimetrically using the blue tetrazolium method. Cortisol levels were compared between ELISA and colorimetric methods, with correlation tests applied to assess the concordance between the two

2.3.3. Validation of system results with ELISA and colorimetry

The system obtained from each participant was immersed in a reactive solution containing blue tetrazolium and buffer solutions, and after color development occurred, the color readings were taken with a smart device equipped with an optical device and a colorimeter. These readings were compared with the results from the ELISA tests, which were used as a reference to assess the accuracy of the colorimeter. In this way, the accuracy of a machine learning-based system in determining cortisol hormone levels using the blue tetrazolium method was evaluated.

While the field study demonstrates a statistically significant correlation between cortisol levels and psychosocial scores, this is not intended to serve as primary validation of the measurement system. The primary validation was conducted through comparison with ELISA and colorimetric reference methods. The observed psychosocial correlations serve only as secondary support, showing the potential real-world relevance of the system, rather than direct biochemical confirmation.

2.3.4. Statistical correlation and success criteria

Pearson and Spearman correlation coefficients are two different correlation measures used in statistical analysis, both of which are used in relational analysis. The Pearson correlation coefficient measures the linear relationship between two variables. This relationship assumes that both variables are normally distributed. Pearson correlation is often used to understand the nature of the relationship between variables. For example, the relationship between two variables may be such that as one variable increases, the other variable also increases or decreases. The Pearson correlation coefficient ranges from -1 to 1. A value of 1 indicates a perfect positive correlation (variables increase together), -1 indicates a perfect negative correlation (one variable increases while the other decreases), and 0 indicates no correlation [17,18,19,20,21]. The formula is as follows:

$$r = \frac{N\sum xy-(\sum x)(\sum y)}{\sqrt{\left[N\sum x^2-(\sum x)^2\right][N\sum y^2-(\sum y)^2]}} \quad (2.12)$$

Where:

N = number of pairs of scores

sum{xy}= sum of the products of paired scores

sum{x} = sum of the x scores

sum{y} = sum of the y scores

sum{x^2} = sum of the squared x scores

sum{y^2} = sum of the squared y scores

The Spearman correlation coefficient measures the relationship between two v ariables, but unlike Pearson correlation, the relationship does not need to be linear. Spearman correlation is used to evaluate the relationship between variables while considering the ranked levels of the data. That is, it examines the relationship between the ranked forms of the data. Spearman correlation is a non-parametric test, meaning that the variables do not need to be normally distributed. The Spearman correlation coefficient also ranges from -1 to 1. However, unlike Pearson correlation, the relationship does not need to be linear [17,18,19,20,21]. The formula is as follows:

$$r_R = 1 - \frac{6\sum_i {d_i}^2}{n(n^2-1)} \quad (2.13)$$

Where:

n = number of data points in both variables

di = difference in the ranks of corresponding variables

The choice of correlation tests depends on dataset size, data distribution, and analysis purpose. Pearson correlation is used for normally distributed data, while Spearman is for non-normal distributions.

Correlation tests assess the statistical significance of the relationship between variables, typically using p-values (14,15,16,17,18). In Python, these analyses are performed with the SciPy library for correlation tests and NumPy for mathematical operations on arrays, facilitating in-depth statistical analysis.

2.4.1. Color readings from the system created with standards and validation with a colorimeter

Color changes were evaluated using a CNN-based system with images captured in different environments and measured with a 3nH colorimeter. RGB values from the colorimeter were compared with ELISA results to assess correlation between color intensity and cortisol concentration. The CNN-based system's results were then validated against both the colorimeter and ELISA, confirming its accuracy.

2.4. Field Application and Correlation Analysis

2.4.1. Participant profile and survey application

Before using the system, participants completed the KOPSOR questionnaire to assess demand, influence, relationships, leadership, job satisfaction, and burnout. Six hours later, the Perceived Stress Scale was administered. Surveys were conducted with 32 foundry employees at Gedik Holding in Sakarya, Turkey. After excluding participants who did not complete the survey or lost/unusable pads, data from 20 participants were analyzed.

2.4.2. Copenhagen psychosocial risk assessment scale

The Copenhagen scale consists of 20 dimensions, each scored on a 1-5 Likert scale, except for job satisfaction, which uses a 1-4 scale. Scores were standardized, and the total score was calculated. Low scores indicate low psychosocial risk, while high scores represent higher risk. The item "Do you work in isolation from your friends?" was reverse-coded. Missing responses were imputed by averaging the participant's scores for the dimension, and participants were excluded if more than 50% of the KOPSOR scale was incomplete [18].

2.4.3. Perceived stress scale

The Perceived Stress Scale (PSS) was scored on a 0-4 Likert scale, with some positive items reverse-scored. The PSS-14 (0-56), PSS-10 (0-40), and PSS-4 (0-16) forms were used, with higher scores indicating higher perceived stress levels [19]. In the Perceived Stress Scale, the scores for the PSS-10 range from 0 to 40, while the scores for the PSS-4 range from 0 to 16 (Table 3.3). A higher score indicates a higher level of perceived stress. Although the current system involves a single sweat sample collected over a six-hour period, the design aims to provide semi-continuous physiological insights rather than instantaneous cortisol readings. While the method does not yet offer real-time or minute-by-minute time-series output, it provides a time-integrated representation of cortisol exposure over a moderate time window.

This approach is not equivalent to continuous cortisol monitoring, such as salivary sampling conducted every 15 minutes. However, it allows for non-invasive, repeated sampling (e.g., every six hours or per work shift), which can be extended into a longitudinal measurement series in practical applications. Importantly, this system enables the tracking of daily, weekly, and monthly cortisol patterns, making it suitable for structured stress surveillance protocols over extended periods. In future iterations, the integration of real-time microfluidic sampling and miniaturized colorimetric sensors may further advance the method toward true continuous monitoring. The six-hour measurement window reflects cumulative stress exposure rather than a specific moment in time. Therefore, the correlation between cortisol levels measured during this window and psychosocial scores represents a first-step validation of the system’s physiological relevance in real-world field conditions.

Although the Perceived Stress Scale (PSS) and Copenhagen Psychosocial Risk Assessment (KOPSOR) reflect longer-term stress states (e.g., over the past week or month), they were used in this study as reference indicators to examine whether the 6-hour cortisol measurements obtained from our system align with broader psychosocial stress patterns. While these scales do not offer real-time precision, the observed correlations provide a preliminary approximation of physiological–psychosocial

consistency. In future studies, repeated cortisol sampling will be synchronized with momentary ecological assessments to improve temporal resolution and strengthen the validity of such comparisons.

3. RESULTS AND DISCUSSION

3.1. Chemical Color Reaction Method

Initially, this method was evaluated on the system. In this stage, 50 µL of tetramethylammonium hydroxide and 80 µL of blue tetrazolium solution were added to a tube, followed by the addition of 10 µL of 1% NaSCN, and then mixed. Subsequently, cortisol stock solutions at appropriate concentrations were added to the prepared solution to form the system. The reaction reached equilibrium after 8 minutes. The resulting colors from the reaction are shown in Figure 3.1.

Figure 3.1. Color Scale Resulting from the Reaction of Stock Solutions Containing 4.5, 6.25, 12.5, 25, and 50 µL/mL Cortisol with the Appropriate Ratios of Color Reagents

3.2. Field Studies

Sweat samples collected from 20 users were analyzed using ELISA, the designed system, and a colorimeter to determine color and cortisol levels for multiple validations. To understand the connection with stress, the Perceived Stress Scale and KOPSOR questionnaire were also administered.

3.2.1. ELISA studies

The absorbance standard curve obtained in the study is shown below (Figure 3.2).

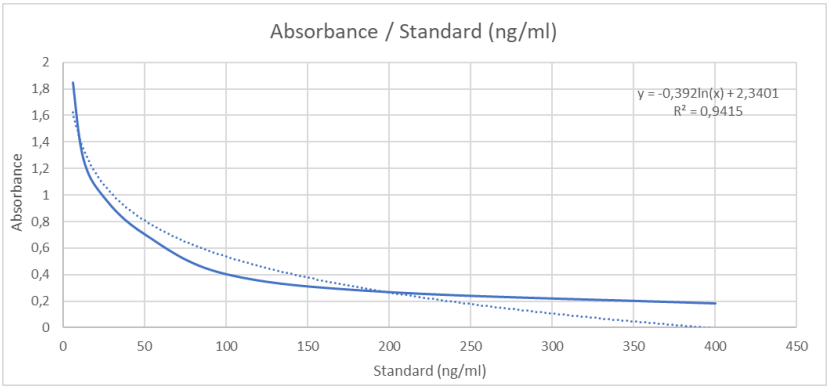

Figure 3.2. ELISA Standard Curve

The absorbance values obtained from the numbered samples on the plate were evaluated using the equation derived from the generated standard curve, and the cortisol hormone level for each user was calculated (Table 3.1, Figure 3.3).

Table 3.1 ELISA Results for Sweat Samples Collected from Users

USERS	ABSORBANCE	CORTISOL HORMONE LEVEL
1	1,647023057	9,686415701
2	1,365487855	11,6835532
3	1,287225142	12,39390801
4	1,50312697	10,61370751
5	1,5038	10,60895731
6	1,191393342	13,3908336
7	1,473847135	10,82456221
8	1,614543578	9,881275559
9	0,6944	22,97487039
10	1,215544184	13,12478
11	1,204120683	13,24929488
12	1,663421307	9,590925603
13	1,456166861	10,9559903
14	1,4777	10,79633891

3.2.2. Survey studies

Before the study, participants completed the KOPSOR questionnaire, which assessed their demands, influence and development desires, interpersonal relationships and leadership status, job satisfaction, and burnout percentages, both individually and collectively.

3.2.2.1. KOPSOR evaluation

Table 4.4 presents the meaning, standard deviation, and median values related to psychosocial risks. When examining participants' conditions in terms of psychosocial risks, subdimensions with high mean values relative to median values indicate high psychosocial risk (Table 3.2).

In this context, the psychosocial risks with high mean values relative to the median include: work pace (57.5±20.74), quantitative demands (40.83±20.75), emotional demands (27.50±26.78), lack of development opportunities (43.13±28.96), role conflict (45.00±30.52), lack of leadership quality (44.38±33.99), lack of social support from superiors (48.75±31.45), lack of sense of community (32.92±30.41), lack of trust (43.44±24.63), lack of job satisfaction (42.30±18.30).

Table 3.2: Central Tendency Measures of KOPSOR-TR Scale Psychosocial Risk Dimensions Scores

	Mean	Standard Deviation	Median	Minimum	Maximum
Work Pace	57,50	20,75	50,00	25,00	100,00
Quantitative Demands	40,83	20,75	41,67	0,00	75,00
Cognitive Demands	69,06	17,96	68,75	37,50	100,00
Emotional Demands	27,50	26,78	25,00	0,00	100,00
Need to Conceal Emotions	43,33	24,27	33,33	16,67	100,00
Lack of Job Influence	58,75	28,42	62,50	0,00	100,00
Lack of Development Opportunities	43,13	28,96	34,38	0,00	93,75

Lack of Job Autonomy	60,31	21,39	65,63	12,50	100,00
Lack of Job Meaning	40,42	36,90	25,00	0,00	100,00
Lack of Job Commitment	39,58	28,34	33,33	0,00	100,00
Lack of Predictability	44,38	32,06	43,75	0,00	100,00
Lack of Recognition	40,83	29,61	41,67	0,00	100,00
Lack of Role Clarity	32,08	28,13	29,17	0,00	100,00
Role Conflict	45,00	30,52	37,50	0,00	100,00
Lack of Leadership Quality	44,38	33,99	37,50	0,00	100,00
Lack of Peer Social Support	47,50	21,31	50,00	0,00	83,33
Lack of Superior Social Support	48,75	31,45	41,67	8,33	100,00
Lack of Sense of Community	32,92	30,41	25,00	0,00	100,00
Lack of Trust	43,44	24,63	43,75	0,00	81,25
Lack of Fairness and Respect	36,88	27,87	40,63	0,00	100,00
Lack of Job Satisfaction	42,30	18,38	43,29	6,66	86,64

3.2.2.2. Perceived stress scale

Table 3.3. Perceived Stress Scale Scores of Employees - PSS Evaluation

	PSS-10	PSS-4
1	18	7
2	14	6
3	21	11
4	15	8
5	17	7
6	26	9
7	17	4
8	6	7
9	16	9
10	15	5
11	31	13
12	7	3
13	13	4
14	17	9
15	3	1
16	28	12
17	11	7
18	19	7
19	13	4
20	18	6

In this analysis (Table 3.4), significant correlations were found between ELISA results and psychosocial risks. In the Job Satisfaction and Burnout dimension, there was a strong positive correlation with ELISA (Spearman: 0.861, $p<0.01$; Kendall Tau: 0.653, $p<0.01$), indicating that higher ELISA values correspond to lower job satisfaction and increased burnout. Similar positive correlations were observed in the Demands dimension (Spearman: 0.645, $p<0.01$), Influence and Development (Spearman: 0.741, $p<0.01$), and Interpersonal Relationships and Leadership (Spearman: 0.638, $p<0.01$), suggesting that higher ELISA values are linked to higher stress and decreased job satisfaction. These findings indicate that ELISA results can influence employees' psychosocial conditions, with a significant impact particularly on job satisfaction, burnout, demands, influence and development, interpersonal relationships, and leadership dimensions.

Table 3.4. Psychosocial risks of participants showing significant correlation with ELISA

	Mean	Standard Deviation	Spearman Correlation Coefficient	p<0,01	Kendall Tau Coefficient	p<0,01
Job Satisfaction and Burnout	44,999	13,499	0,861	1.05×10^{-6}	0,653	2×10^{-5}
Demands	47,646	13,014	0,645	2.15×10^{-3}	0,480	3×10^{-3}
Influence and Development	48,438	17,787	0,741	1.8×10^{-4}	0,568	2.7×10^{-4}
Interpersonal Relationships and Leadership	41,615	17,751	0,638	2.49×10^{-3}	0,442	5.92×10^{-3}
Job Satisfaction	42,299	18,379	0,728	2.7×10^{-4}	0,557	8.9×10^{-4}

Table 3.5. Table Showing the Main Reference Values' Concentration, L, a, b Values, Lab Average, RGB Values, RGB Average, and ELISA Absorbance

Concentration	L	A	B	Lab Mean	R	G	B	RGB Mean	Elısa
6,25	66,7	14,9	0,4	27,33	189	153	162	168,00	1,8454
12,5	61,6	21,7	-1,5	27,27	185	135	152	157,33	1,2763
25	52,6	28,5	-5,7	25,13	168	107	136	137,00	0,9851
50	48,4	30,7	-7,3	23,93	159	95	128	127,33	0,7036
100	40,5	37,2	-4,1	24,53	148	69	103	106,67	0,4016
200	34,9	34,2	-10,1	19,66	126	59	99	94,67	0,2662
400	27,5	39,7	-1,7	21,83	117	33	69	73,00	0,1803

Table 3.5 includes measurements of concentration, L, a, b values, Lab and RGB averages. As concentration increases, both ELISA absorbance and RGB values decrease, indicating an inverse relationship between concentration and absorbance or color intensity. The L value reflects brightness, while a and b define hue and saturation. Changes in these values with concentration help determine color differences among samples.

Table 3.6: Cortisol hormone levels obtained from employees via ELISA and RGB values obtained from the system using a colorimeter

USERS	CORTISOL HORMONE LEVEL	AVARAGE RGB
1	9,686415701	146,3333
2	11,6835532	149,6667
3	12,39390801	153
4	10,61370751	146,6667
5	10,60895731	146
6	13,3908336	153,6667
7	10,82456221	144

8	9,881275559	147
9	22,97487039	174,6667
10	13,12478	149,6667
11	13,24929488	148
12	9,590925603	137,3333
13	10,9559903	141,6667
14	10,79633891	140
15	10,12456221	139,3333
16	11,12757029	147,6667
17	13,99329006	154
18	10,77228224	149,3333
19	14,20732267	155,6667
20	11,57380037	147,3333

In Table 3.6, cortisol levels typically range between 9 and 14 units, except for the 9th employee, who has a notably higher cortisol level of 22.97 units, suggesting high stress or other factors. RGB values generally range from 139 to 155, but the 9th employee's average is significantly higher at 174.67. This variation may reflect differences in the work environment. The data shows a consistent relationship between cortisol levels measured by ELISA, absorbance values, and average RGB values, with higher cortisol levels corresponding to lower absorbance and higher RGB values.

Below, the results of the normality test conducted on the data presented in Table 3.7: Cortisol Hormone Levels Obtained from Employees via ELISA and RGB Values Obtained from the System Using a Colorimeter are provided (Table 3.7).

Table 3.7: Normality tests for cortisol hormone levels obtained via ELISA and average RGB values obtained with a colorimeter within the system

Variable	**Shapiro-Wilk Statistic**	**p-value**
Cortisol Hormone Levels	0.6823	2.39e-05
Avarage RGB	0.8432	0.0041

The p-values for both variables are below 0.05, indicating non-normal distribution, so the Spearman correlation was used. The Spearman correlation coefficient is -0.987 ($p<0.005$), showing a strong negative relationship between the two variables, meaning as one increases, the other decreases. The p-value of 0.0 confirms statistical significance. This demonstrates a strong negative correlation between cortisol levels in sweat samples and ELISA absorbance, consistent with the study's objectives.

Table 3.8. Correlation tests (spearman analysis) for cortisol hormone levels obtained via ELISA and average RGB values obtained with a colorimeter within the system for each user.

Users	**Independent Variable: Cortisol Hormone Level (ng/mL**	**Dependent Variable: Average RGB**	**Spearman Correlation Coefficient**
1	9,686	146,333	*-0.90
2	11,684	149,667	*-0.95

3	12,394	153,000	*-0.97
4	10,614	146,667	*-0.92
5	10,609	146,000	*-0.89
6	13,391	153,667	*-0.94
7	10,825	144,000	*0.75
8	9,881	147,000	*0.81
9	22,975	174,667	*-0.67
10	13,125	149,667	*-0.90
11	13,249	148,000	*-0.90
12	9,591	137,333	*0.71
13	10,956	141,667	*-0.82
14	10,796	140,000	*-0.82
15	10,125	139,333	*-0.78
16	11,128	147,667	*-0.96
17	13,993	154,000	*-0.99
18	10,772	149,333	*-0.93
19	14,207	155,667	*-0.95
20	11,574	147,333	*-0.96

*p<0.001

Table 3.8 shows a strong negative Spearman correlation between cortisol levels and average RGB values, indicating that as cortisol levels increase, RGB values decrease. This inverse relationship is statistically significant ($p<0.01$) for all employees.

Table 3.8 presents the Spearman correlation coefficients between cortisol hormone levels measured via ELISA and average RGB values obtained from the colorimetric system for each participant. The results reveal a consistently strong negative correlation across many subjects, with coefficients generally ranging between -0.78 and -0.99, and all being statistically significant ($p < 0.001$). This indicates that as cortisol concentration increases, the average RGB value derived from the pad's color decreases, which is in line with the expected outcome of the blue tetrazolium reaction — higher cortisol concentrations result in darker color development.

Interestingly, a few outliers (e.g., Users 7, 8, and 12) exhibit positive correlations, which may be due to sample anomalies, measurement deviations, or individual physiological variability. Despite these exceptions, the overall pattern supports the robustness of the colorimetric system in reflecting cortisol concentrations.

These findings validate the inverse relationship between cortisol levels and RGB color intensity, highlighting the potential of the blue tetrazolium-based image analysis method as a practical alternative to ELISA for non-invasive, rapid stress monitoring. The strong correlations across most participants support the feasibility of implementing the system for real-world applications in occupational or clinical settings.

These findings suggest that evaluating cortisol levels alongside color values could effectively monitor stress or biological factors. The blue tetrazolium colorimetric method shows high accuracy in determining cortisol levels and could serve as a reliable alternative to the ELISA method.

3.3.1. Determining Cortisol Hormone Levels Using Machine Learning on Images Captured by Smart Devices

The lack of correlation in the data obtained without the image processing process (Figure 3.3) marked the starting point of this study. To standardize the data, image processing techniques such as saturation, contrast, light intensity, white balance, pixel values, and resizing were applied to the images.

The table below (Table 4.10) presents the analysis of images taken from different optical devices in various environments before image processing.

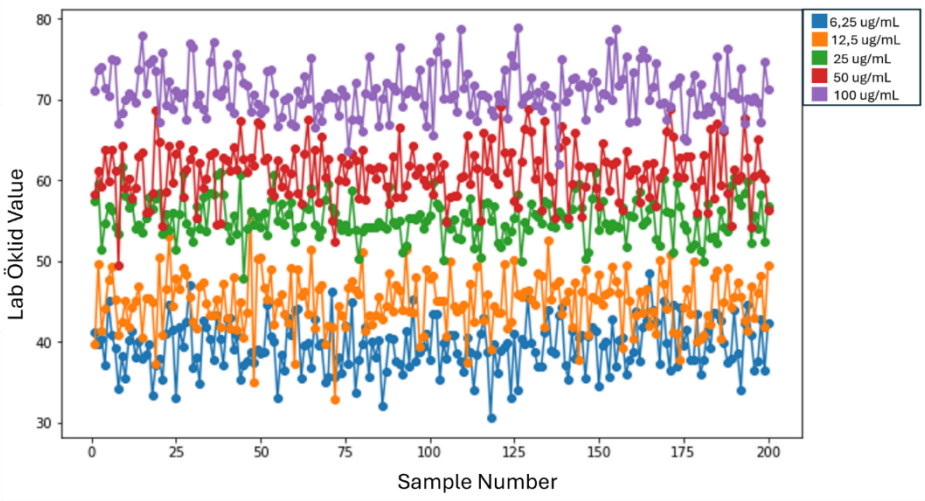

Figure 3.3. Comparison of lab Euclidean values obtained from unprocessed images for each concentration

Table 3.9. Analysis results of images taken in different environments with devices featuring different optical equipment

Reference Concentration	Sample Size (n)	Min	Max	Range	Skewness	Kurtosis	Standard Deviation	Coefficient of Variation	Pearson Correlation Coeffient	Pearson p value
6,25	200	30,61	48,47	17,86	0,044	-0,148	3,113	7,890	0,106	0,140
12,5	200	32,94	56,19	23,25	-0,128	0,533	3,469	0,072	0,004	0,952
25	200	47,83	61,74	13,91	0,116	-0,025	2,532	0,045	-0,081	0,253
50	200	49,54	69,21	19,67	-0,092	0,088	3,439	0,056	0,033	0,643
100	200	62,05	78,84	16,79	0,305	0,147	2,990	0,042	-0,047	0,502

In the dataset analysis (Table 3.9), samples with varying concentrations were evaluated using statistical measures. Across concentrations (6.25, 12.5, 25, 50, 100 µg/mL), datasets are right-skewed and non-normal. Standard deviations range from 2.53 to 3.47, and coefficients of variation generally decrease with higher concentrations, except for a slight increase at 100 µg/mL. Pearson correlation coefficients are weak and statistically insignificant across all concentrations. For the 6.25 µg/mL concentration, the range is 6.27, skewness is 0.515 (right-skewed), and kurtosis is -3.154 (flat). The standard deviation is 3.13, indicating data variability. Variations in lighting and resolution contribute to the non-linear relationships, emphasizing the need for image processing to standardize data for machine learning.

3.3.2. Cortisol hormone determination using computer vision

In this study, image processing applications were used to enhance images, and machine learning methods were employed to develop system training and prediction models.

3.3.2.1. Image Processing

Color intensity calculations were performed on raw images, brightness-corrected images, and images processed through image processing techniques. Intra-category correlation variation was determined using standard deviation differences. The stability of the images and colors after applying image processing algorithms is shown in Figure 3.4.

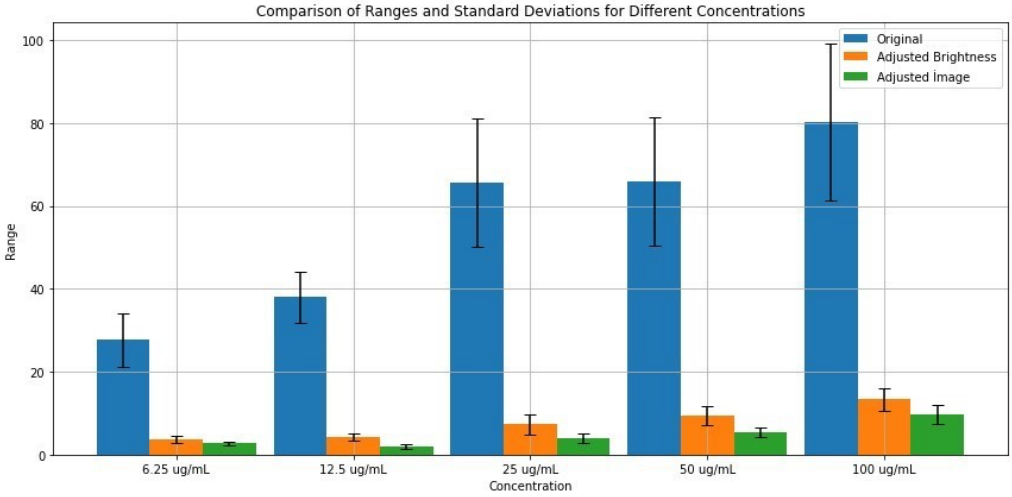

Figure 3.4. Graph showing the impact of image processing on image quality and color intensity values

The standard deviation and range of average RGB values for unprocessed images are as follows: 6.25 µg/mL (6.48-27.67), 12.5 µg/mL (6.25-38), 25 µg/mL (15.4), 50 µg/mL (14.48-65.67), and 100 µg/mL (18.9-80.33). These values show high variability. After brightness processing, the standard deviations and ranges decreased: 0.96-13.33 across concentrations, indicating reduced variability. With full image processing, the standard deviation further normalized: 0.56-9.67. Image processing significantly reduced variability, resulting in more standardized data suitable for machine learning.

3.3.2.2. Outputs from convolutional neural networks

The model obtained from the training process demonstrated high accuracy and good generalization ability, indicating that its ability to determine concentrations is reliable. The model's performance was evaluated on the test dataset, yielding successful results.

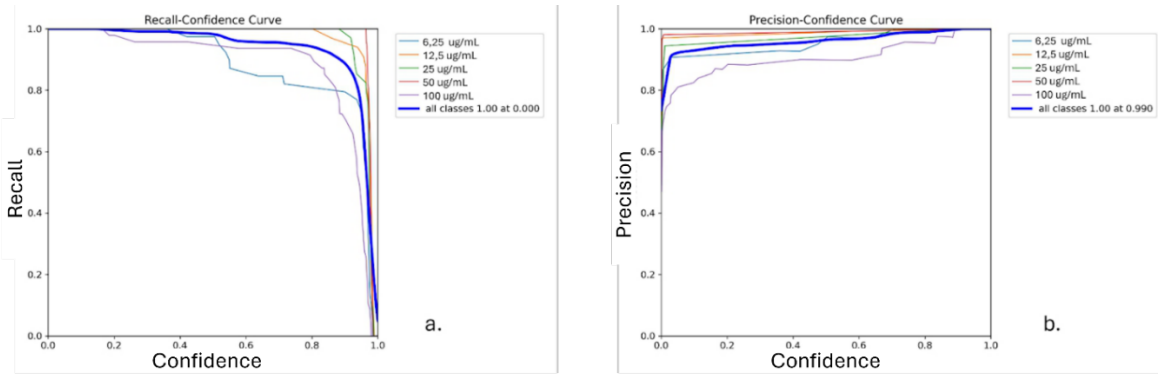

Figure 3.5. (a) Sensitivity-Confidence (b) Precision-Confidence

Sensitivity-Confidence Graph: (Figure 3.5) This graph shows Sensitivity values plotted against Confidence values. As Sensitivity values increase, Confidence values also increase. For all classes, Sensitivity values reach 1.00 at a Confidence level of 0.000. Precision-Confidence Graph: In this graph,

Precision values are plotted against Confidence values. As Precision values increase, Confidence values also increase. For all classes, Precision values reach 1.00 at a Confidence level of 0.990.

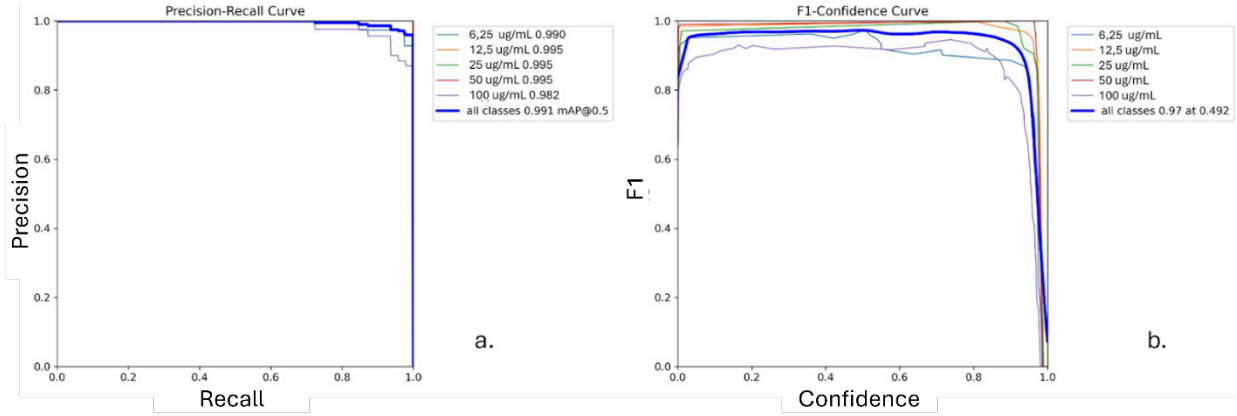

Figure 3.5. (a) Precision-Recall Graph (b) F1-Confidence Graph

Precision-Recall Graph: (Figure 3.5) This graph shows Precision values plotted against Recall values. As Precision values increase, Recall values also increase. The mAP@0.5 value for all classes is 0.991. F1-Confidence Graph: The F1 score for all classes is 0.97 at a Confidence level of 0.492. This indicates that the model performs well with both high accuracy and recall.

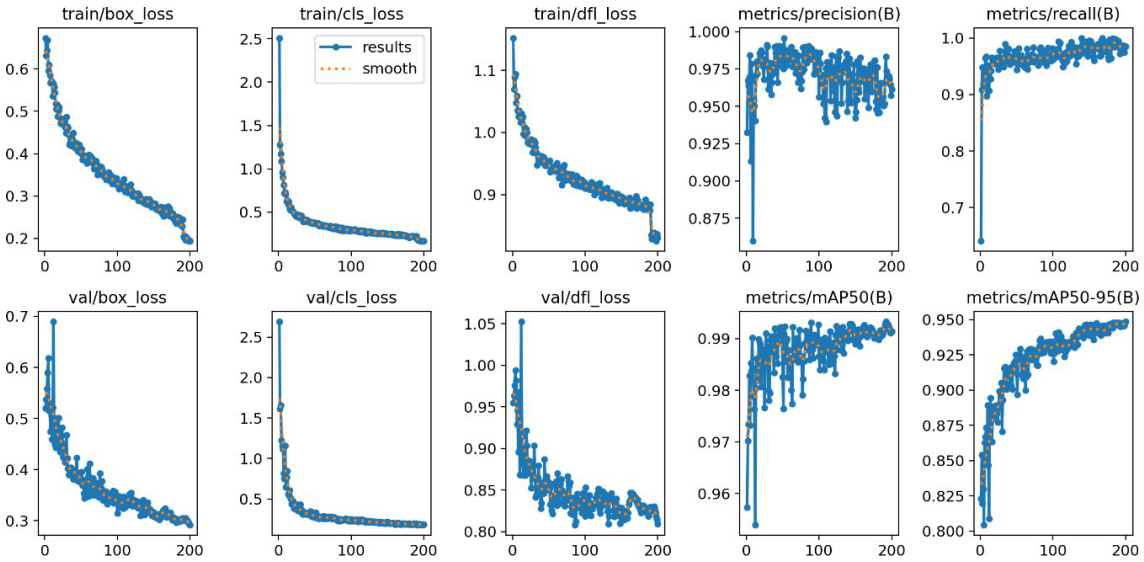

Figure 3.6. Graphs Showing the Training Process of the Machine Learning Model

Figure 3.6 illustrates various performance metrics during the training process of the proposed model:

- a. Bounding Box Loss: Shows a decreasing trend in both training and validation, indicating improved accuracy in object detection.

- b. Class Loss: Reflects decreasing classification loss for both training and validation, showing better classification accuracy.

- c. Total Loss: Combines bounding box and class loss, with a downward trend, indicating overall model performance improvement.

- d. Precision and Recall: Both metrics improve for class B, showing better accuracy in positive predictions.

- e. mAP50: Mean Average Precision for class B increases, showing better overall model accuracy at an IoU threshold of 50%.

These graphs indicate the model's steady improvement in performance, with decreasing loss and increasing accuracy, precision, and recall.

3.3.2.3. Regression-based prediction model

A vector regression model was trained using image values to predict cortisol hormone levels, followed by decision tree regression to segment and group employees by similar characteristics. These methods improved both accuracy and interpretability. The regression-based prediction algorithm used image data from participants, though interpolation predictions were less successful than reference training. Prediction results and success percentages are shown in Tables 3.10-3.11.

Table 3.10. System Predictions and Prediction Success Rate

Users	**Cortisol Hormone Level**						**Success Rate %**	
	ELİSA	**Prediction**	**Standard Deviation**	**Mean**	**Varience**	**Range**	**MAE**	**RMSE**
1	9,69	12,78	1,55	11,23	2,39	3,09	85,55	81,05
2	11,68	10,05	0,82	10,87	0,67	-1,63		
3	12,39	10,65	0,87	11,52	0,76	-1,74		
4	10,61	11,67	0,53	11,14	0,28	1,06		
5	10,61	11,99	0,69	11,30	0,48	1,38		
6	13,39	11,76	0,82	12,58	0,66	-1,63		
7	10,82	11,97	0,57	11,40	0,33	1,15		
8	9,88	9,02	0,43	9,45	0,19	-0,86		
9	22,97	15,20	3,89	19,09	15,11	-7,77		
10	13,12	14,87	0,87	14,00	0,76	1,75		
11	13,25	14,92	0,84	14,08	0,70	1,67		
12	9,59	8,64	0,48	9,12	0,23	-0,95		
13	10,96	11,94	0,49	11,45	0,24	0,98		
14	10,80	11,33	0,27	11,06	0,07	0,53		
15	10,12	11,78	0,83	10,95	0,69	1,66		
16	11,13	12,79	0,83	11,96	0,69	1,66		
17	13,99	12,10	0,95	13,05	0,90	-1,89		
18	10,77	11,58	0,40	11,18	0,16	0,81		
19	14,21	15,68	0,74	14,94	0,54	1,47		

20	11,57	10,34	0,62	10,96	0,38	-1,23

Table 3.11 shows that the model successfully predicted cortisol levels for some samples, such as 8 and 13, where predicted values closely matched actual ELISA results with low error. However, in cases like sample 9, predictions significantly differed from actual values, with higher error and standard deviation. Overall, the model's accuracy varied, indicating the need for further improvement and more data for better predictions.

Table 3.11: Correlations Between System Predictions and Psychosocial Assessments

	Dependent Variable	**Independent Variable**		**PEARSON (P<0,05)**	
Users	**Prediction**	**Demands**	**Job Satisfaction**	**Prediction-Demands**	**Prediction-Job Satisfaction**
1	12,78	42,08	20,00		
2	10,05	48,33	59,96		
3	10,65	35,00	53,28		
4	11,67	32,92	33,30		
5	11,99	44,58	46,62		
6	11,76	57,92	46,62		
7	11,97	30,83	33,30		
8	9,02	31,67	6,66		
9	15,20	76,67	66,64		
10	14,87	51,67	86,64		
11	14,92	68,33	46,62	0,66	0,53
12	8,64	27,08	13,32		
13	11,94	48,33	46,62		
14	11,33	45,83	33,30		
15	11,78	55,00	33,33		
16	12,79	65,00	33,30		
17	12,10	55,42	59,94		
18	11,58	47,50	46,62		
19	15,68	46,25	39,96		
20	10,34	42,50	39,96		

Prediction (Cortisol Level): The cortisol levels predicted from employees' sweat samples.

The Shapiro-Wilk test confirmed that the data for predicted cortisol levels, job demands, and job satisfaction were normally distributed ($p > 0.05$), justifying the use of Pearson correlation analysis. As shown in Table 3.11, a moderate positive correlation was found between predicted cortisol levels and job demands ($r = 0.66$, $p < 0.05$), as well as between cortisol and job satisfaction ($r = 0.53$, $p < 0.05$).

The strong association between high job demands and elevated cortisol levels suggests that occupational workload is a primary driver of physiological stress responses, consistent with established findings in workplace stress literature. However, the observed positive correlation between job satisfaction and cortisol levels is less intuitive. It may reflect a scenario where employees feel fulfilled in their roles yet still operate under intense pressure or responsibility, as commonly seen in leadership or high-performance positions. This aligns with studies indicating that satisfaction does not always equate to reduced physiological stress, particularly in demanding roles.

These findings reveal the complex and multifactorial nature of workplace stress, emphasizing that both negative (e.g., excessive workload) and positive (e.g., meaningful engagement) psychosocial dimensions can elicit stress-related hormonal responses. Therefore, occupational health interventions must go beyond reducing dissatisfaction—they must also manage workload and foster sustainable engagement to balance satisfaction with well-being.

4. CONCLUSIONS

The study evaluates the effectiveness of methods for determining stress levels, comparing ELISA with a portable system. ELISA, though accurate, requires lab settings and takes hours, making it impractical for continuous monitoring. The designed system offers a quicker, user-friendly alternative using portable sensors and colorimetry, providing rapid stress analysis and feedback. It automates sweat sample collection and reaction analysis, making it ideal for continuous monitoring, particularly for individuals with mobility issues.

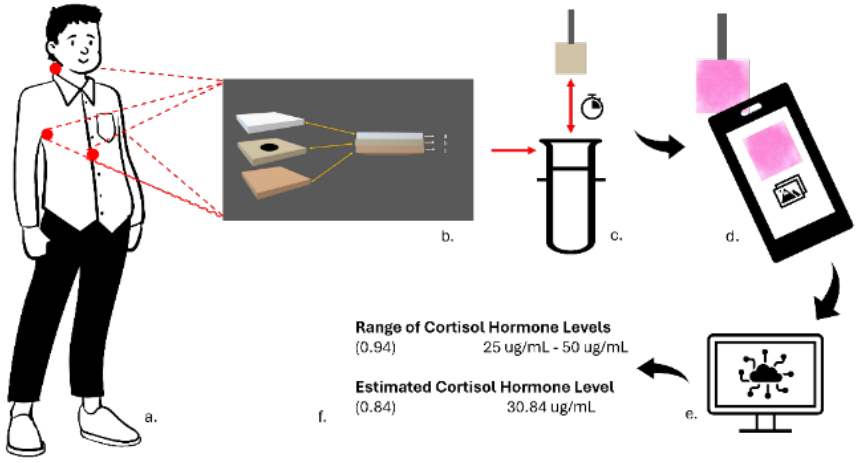

Figure 4.1. Operating Principle of the Proposed System

Microfluidic pads placed in sweat collection areas (a, b) remain on the body for 6 hours. Afterward, the pads are immersed in a blue tetrazolium color reagent for 3 minutes (c). The color change on the pad is photographed using a device with optical capabilities (d). The image is sent to the system (e), where it is processed using image processing algorithms and evaluated within prediction models before being displayed on the results screen (f).

The study compares ELISA with a newly designed portable system for stress monitoring. While ELISA is accurate in labs, the designed system offers portability and quicker analysis, but requires further validation. Future research is needed to assess the system's advantages over traditional methods.

Other studies, like Tu et al. (2020), explored various colorimetric methods for cortisol detection, with the blue tetrazolium method proving most effective. Similarly, a 2023 study by Fiore et al. developed a microfluidic device using magnetic beads and NFC for cortisol detection in sweat, showing promising results. These findings highlight the potential of portable, real-time stress monitoring technologies, encouraging collaboration between healthcare and technology sectors for advanced stress management solutions (26,27,28)

This study validated the reliability of a sustainable paper-based device for cortisol detection during cycling activities, highlighting the effectiveness of wearable immunosensors and microfluidic devices. These portable, low-cost, non-invasive tools show potential for stress monitoring, complementing traditional methods like ELISA. Future research should focus on further validating these systems for real-world applications.

The study also examined correlations between cortisol levels (measured by ELISA) and psychosocial risks assessed by the KOPSOR questionnaire and perceived stress scale. High cortisol levels were linked to low job satisfaction, increased stress, and negative psychosocial conditions such as high demands, burnout, and poor leadership. These findings emphasize the role of cortisol in assessing workplace psychosocial risks, contributing to the development of occupational health policies.

The AI-supported colorimetric system developed in this study offers a fast, portable, and user-friendly solution for the detection of cortisol in sweat samples. The system analyzes color changes using blue tetrazolium (BT) dye and thus determines stress levels. This approach is similar to the work of Ahmed et al. with the Salitrack sensor; the sensor in question detected cortisol levels in artificial saliva samples using BT dye with accuracy comparable to ELISA (51)

Fiore et al. developed a microfluidic paper-based electrochemical biosensor and detected cortisol in sweat using magnetic beads and NFC technology (28). This system attracts attention with its portability and stability up to 28 days. Similarly, the system developed in our study also offers the advantages of portability and rapid analysis for the detection of cortisol in sweat samples.

However, aptamer-based sensors are also promising for the detection of cortisol. In a study published in 2024, a low-cost and rapid method for detecting cortisol using aptamers functionalized with gold nanoparticles was developed (50). This approach can provide a strong basis for the integration of aptamer-based detection mechanisms in future versions of our system.

As a result, the developed AI-supported colorimetric system offers advantages such as portability, rapid analysis and user-friendly interface compared to similar studies in the literature. These features make the system a suitable tool for monitoring stress levels in workplace environments and field applications.

In conclusion, this study highlights the benefits of using biological markers like ELISA to identify psychosocial risks and manage workplace stress. However, larger-scale studies are needed to validate these findings and explore integration into workplace practices. Future research should focus on understanding workplace stress and developing strategies to improve employee health.

Nordholm et al.'s 2023 study also examined the link between cortisol levels and work stress using the ELISA method. The findings indicate that higher work stress correlates with increased cortisol levels, with differences observed among occupational groups. These results underscore the potential of ELISA in studying the effects of work stress on health and managing workplace stress effectively (29).

In H. Janssens et al.'s 2017 study, a questionnaire gathered data on socio-demographics, health behaviors, work stress, and depression symptoms, with cortisol levels measured in hair samples using the ELISA method. The Job Content Questionnaire assessed work stress, while the Copenhagen Psychosocial Questionnaire evaluated psychosocial risks like emotional and cognitive demands. Depression symptoms were measured with the short Iowa version of the CES-D scale. Health behaviors, such as smoking and alcohol use, were included as confounding variables.

The study revealed high psychosocial risks in areas such as work speed, demands, and lack of development opportunities, with positive correlations between cortisol levels and factors like job satisfaction, burnout, and leadership. High cortisol was linked to increased stress and negative work

conditions, highlighting the role of ELISA in assessing psychosocial risks and workplace stress. Further research with larger samples is needed to confirm these findings and develop effective stress management strategies [30].

In this study, two methods were used to monitor workplace stress: cortisol levels measured via ELISA and colorimetrically determined average RGB values to assess stress visually. Statistical analysis, including Pearson and Spearman correlations, showed a strong negative relationship between cortisol levels and RGB values, both statistically significant. This suggests that stress-induced cortisol changes can be effectively monitored using colorimetric measurements, making it a potential tool for workplace stress management.

Similarly, Pham et al.'s 2021 study developed a method for stress monitoring using ELISA and concave lateral flow immunoassay (cLFIA) test strips, demonstrating that colorimetric measurements are faster, more sensitive, and less costly than traditional lab techniques. These methods could play a crucial role in managing workplace health and stress levels [24]. The study also utilized RGB (Red, Green, Blue) values for color analysis. Images of cLFIA test strips, taken with an iPhone 7, were analyzed in the RGB color space, focusing on the intensity of the green channel to determine cortisol levels. This method offers a visual approach to detecting stress levels through colorimetric signals [31].

Yang M. et al.'s 2023 study focused on cortisol detection using ELISA, which accurately measures cortisol levels through the binding of cortisol molecules to specific antibodies. The study combined ELISA with RGB color analysis, improving the accuracy of colorimetric signals and demonstrating fast, low-cost cortisol detection, useful for early treatment of stress-related health issues [32].

Both Yang et al. and Pham et al. examined methods for monitoring workplace stress through cortisol detection. Pham integrated ELISA with colorimetric measurements, finding a strong negative correlation between cortisol levels and RGB values, while Yang emphasized a sweat-based diagnostic device using RGB analysis. This study confirmed the reliability of colorimetric analysis compared to ELISA results.

Colors from the high-accuracy system for reference concentrations (6.25, 12.5, 25, 50, 100 μg/mL) were photographed using different devices, and RGB values were determined. Data analysis showed that as concentration increased, standard deviation and coefficient of variation varied, but no linear relationship was found between variables and concentration in any sample. Image processing techniques improved accuracy by correcting saturation, contrast, and lighting issues, leading to more consistent results when analyzed with machine learning.

The primary goal of the project is to measure occupational stress objectively, helping prevent workplace accidents and improve efficiency. However, cortisol levels can fluctuate due to medications and diseases like hypertension, obesity, and diabetes, affecting measurement reliability. Personalized data collection is needed to improve accuracy, and future studies should consider a more comprehensive approach, including individuals with different health conditions.

This system can be used not only for cortisol hormone determination but also for all parameters that can be analyzed colorimetrically, providing quantitative results. This situation further enhances the applicability and advantages of systems supported by artificial intelligence algorithms. Thus, rapid, reliable, and objective analyses can be performed for a wide range of biomarkers, allowing for significant progress in health monitoring and evaluation processes.

Declaration of Ethical Standards

It was approved by the Gedik University Ethics Committee Commission with the ethics committee decision numbered E-56365223-050.01.04-2022.137548.146.

Credit Authorship Contribution Statement

Muhammed Ertuğrul Çapan and Ebru Cingöz Çapan contributed substantially to the conception and design of the study. Methodology development, data acquisition, formal analysis, and data curation were primarily performed by Muhammed Ertuğrul Çapan and Ebru Cingöz Çapan. Software development,

image processing pipeline design, and artificial intelligence–based analysis were carried out by Muhammed Ertuğrul Çapan, while Ebru Cingöz Çapan contributed to validation and interpretation of the analytical results. The original draft of the manuscript was written by Muhammed Ertuğrul Çapan and Ebru Cingöz Çapan, and both authors led the revision and editing process. Visualization and figure preparation were mainly undertaken by Muhammed Ertuğrul Çapan with contributions from Ebru Cingöz Çapan. Hasan Uğur Öncel and Ercan Arıcan contributed through critical review, methodological supervision, and scientific control of the study. They provided expert feedback during the validation and final evaluation stages of the manuscript. All authors reviewed and approved the final version of the manuscript.

Declaration of Competing Interest

There is no conflict of interest between the authors.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. The dataset used in this study includes colorimetric readings, ELISA measurements, and psychosocial risk assessment results collected from participants. Due to privacy concerns and ethical considerations, raw data will be shared only for academic and research purposes upon approval.

Use of Artificial Intelligence

Artificial intelligence tools embedded in word processing software were used solely to improve language quality, grammar, and readability of the manuscript. The authors take full responsibility for the accuracy, originality, and scientific integrity of the content of this article. The use of artificial intelligence tools was strictly limited to language editing and readability improvements, in accordance with ethical and editorial guidelines.

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