

Infrared Thermography as a Non-Invasive Tool to Assess Health Bio-Marks in University Students

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ABSTRACT

Aim: To evaluate the relationship between cortisol concentrations (ng/ml) with body temperature, digital thermometer and thermal infrared gun (°C). Infrared Thermography is a new and revolutionary way of measuring body temperature.

Methodology: In this study data is collected about 65 individuals and extract blood samples from 50 students (25 males and 25 females).

Results: Non-significant negative correlation was observed between cortisol concentrations (ng/ml) with body temperature of IRT of right eye-maximum (CC = -0.100; P = 0.490). Non-significant negative correlation was observed between cortisol concentrations (ng/ml) with body temperature of IRT of left eye-maximum (CC = -0.099; P = 0.495). Non-significant negative correlation was observed between cortisol concentrations (ng/ml) with body temperature of IRT of forehead-maximum (CC = -0.124; P = 0.413). A non-significant negative correlation is also observed between cortisol concentration (ng/ml) with body temperature of IRT of mean of left eye, thermal infrared gun and digital thermometer (°C).

Conclusion: Overall non-significant negative correlation is also observed between various concentrations with body temperature.

Keywords: IRT, Cortisol, Body temperature, Digital thermal temperature, Infrared gun temperature

INTRODUCTION

Infrared Thermography is a technique which uses infrared radiation to see thermal energy emitted by the body. A thermographic camera records the surface temperature as well as the heat pattern within the body. These infrared radiations are connected with human skin temperature and calculated through IRT¹. Thermal imaging can capture the human body radiations and change into signalize form². The temperature has become a good predictor of health in the past³. Thermometers were invented in the seventeenth century⁴.

The human body temperatures ear and forehead regions are calculated through non-invasive infrared thermal cameras to treat disorders. Infrared thermal cameras work very rapidly, and they are easy to use. Medicinal Thermal cameras measure tympanic and forehead temperature and they are beneficial in medical science⁵⁻⁶. Infrared thermography can be used for various applications such as detecting human body temperature changes, monitoring skin color, identify skin diseases and prevent sunburns⁷.

IRT and Thermostats are immediate and non-invasive tools to measure the temperature of human body skin. Clinical thermometers and infrared thermography permit nearly instantaneous and non-contact temperature measurements, whereas they calculate human skin temperature⁸. The accurateness of non-invasive temperature dimensions can be enhanced by using a double belt or manifold thermal analysis⁹⁻¹⁰.

Cortisol decrease the function of brown fatty tissue (BAT) in animals, making them more prone to obesity¹¹⁻¹². There is a scarcity of data on how glucocorticoids affect BAT function in people¹³. The temperature of the core of the body is linked to the state of the environment¹⁴. The efficacy of laboratories

psychological stress tests to accurately obtain salivary cortisol reactions varies¹⁵. Many patients are dying due to blood pressure, and this disorder creates many colonial issues in this world. Blood pressure is a severe illness of heart disease, and the patient feels sick¹⁶.

The objective of this paper was to find how cortisol concentration level significantly correlates with body temperature and find significant correlation between temperature of infrared gun (°C), digital thermometer temperature (°C), and thermal infrared temperature.

METHODOLOGY

Experimental Data: From July 2021 the study trial was organized at the Baghdad-all-jaded site of Islamia institute of Bahawalpur for four days. Entire 65 people duration 18-31 year have partaken in this evaluating trial. Out of these 65 students 35 were male and 30 were female. And blood was taken from 50 students in which 25 were males and 25 were females. We measure different body parameters like body temperature (through Infrared Thermographic camera, Infrared gun and Digital Thermometer in Celsius) during day time at 10 AM to 2 PM.

Infrared Thermogram Analysis: Thermographic images of the face had been received through the use of a Fluke PTi 120 Thermal Imagers (Fluke Corporation, Everett, WA, USA). The infrared thermographic camera has a stable attention, lower limit space is 22.8 cm, it operates with inside the 8 to 14µm wavelength variety, with ±2°C accuracy and a thermal sensitivity (NETD) ≤ 60mk. The digital digicam works withinside the variety of -4 to 302°F (-20 to 150°C). The marker become extensively utilized to pick out the location of hobby with inside the processing phase to ensure that the IRT digital digicam become located on the identical distance from the issue each time.

Infrared Thermographic Camera: During research all students were sitting in laboratory for 15 minutes where temperature and humidity were controlled and the influence of surrounding

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temperature on the skin was minimum. Thermal infrared camera was put on a three-legged stand, and subject was sitting on chair one meter distance from the camera for capturing thermal images. Infrared thermal camera was focused for 60 seconds on the region of interest (ROI) and IR thermograms were obtained: (1) forehead (2) eyes. Two thermogram of each subject were taken one before getting the blood and other after taking the blood.

Figure 1. Infrared Thermographic Camera (FLUKE PTi 120)



Thermal Imaging before Sample Collecting: Blood samples collected from experimental data are used for Cortisol test. The thermal camera was positioned to the front of the face at one meter distance focusing the forehead and eyes of the students. The first thermogram was taken before taking the blood sample. Venipuncture sampling method was adopted. The blood was taken from a peripheral blood vessel, in the upper arm, commonly the median basilica blood vessel through 5cc syringe. The blood was collected into EDTA tubes. After blood collection of blood the second thermogram was captured.

Blood Serum Isolation: Blood serum is blood plasma that has been stripped of its clotting components. In the Appendorf (centrifuge) tube, clotted blood resolved into three components erythrocyte, fibrin-platelets, and neutrophils are transmitted, we centrifuge (Model 800) for 15 minutes at 2200-250 RPM within 60 minutes of accumulation. Transfer the serum to moldable Appendorf tubes with a clean pipette for freezing to avoid any bacterial activity.

Figure 2. Blood Serum Isolation and measurement of humidity



Digital Thermometer an Infrared Temperature Gun: Before temperature perusing ensure the subject was prepared to test by

ensuring that the test area of the forehead is clean, dry and unhindered. Infrared temperature gun placed perpendicular to the person forehead and readings were measured in degree Celsius.

Statistical Analysis: All analyses were conducted using Minitab 18. Mathematical models of regression were also calculated through Minitab. Values were expressed as mean standard deviation and statistical significance was considered as p-value (<0.05).

Table 1: Measurement values of 50 individuals to find correlation between Cortisol concentrations (ng/ml) with different body temperature (°C)

| No. of Individuals | Temperature of Infrared Gun (°C) | Digital thermometer Temperature (°c) | Cortisol conc (ng/ml) |
|--------------------|----------------------------------|--------------------------------------|-----------------------|
| Individual 1 | 36.7 | 35 | 199.9985246 |
| Individual 2 | 37.2 | 36.44 | 203.5070691 |
| Individual 3 | 36.8 | 36.66 | 199.7479142 |
| Individual 4 | 36.5 | 36.44 | 204.8854259 |
| Individual 5 | 36.3 | 35.05 | 213.1555667 |
| Individual 6 | 36.8 | 35.88 | 209.3964118 |
| Individual 7 | 36.5 | 34.72 | 238.71782 |
| Individual 8 | 36.2 | 36.6 | 236.7129374 |
| Individual 9 | 37.4 | 36.5 | 211.6519047 |
| Individual 10 | 36.6 | 36.44 | 200.6250504 |
| Individual 11 | 36.9 | 37.16 | 178.9472571 |
| Individual 12 | 36.5 | 36.83 | 206.5143931 |
| Individual 13 | 36.4 | 36.44 | 193.4826561 |
| Individual 14 | 36.5 | 36.83 | 213.1555667 |
| Individual 15 | 36.4 | 34.83 | 194.2344871 |
| Individual 16 | 36.5 | 34.66 | 247.2385711 |
| Individual 17 | 36.9 | 37.16 | 220.0473507 |
| Individual 18 | 37.1 | 36.77 | 254.0050499 |
| Individual 19 | 36.6 | 36.66 | 201.878102 |
| Individual 20 | 36.6 | 37.33 | 122.1840182 |
| Individual 21 | 36.6 | 36.66 | 204.8854259 |
| Individual 22 | 36.5 | 36.83 | 222.6787591 |
| Individual 23 | 36.6 | 34.94 | 237.0888529 |
| Individual 24 | 36.5 | 37.1 | 240.0961768 |
| Individual 25 | 36.7 | 37.38 | 239.324675 |
| Individual 26 | 36.4 | 37 | 260.2703081 |
| Individual 27 | 37 | 36.55 | 261.6486649 |
| Individual 28 | 36.8 | 36.16 | 200.1238297 |
| Individual 29 | 37.3 | 36.11 | 208.8951912 |
| Individual 30 | 37 | 36.55 | 225.9366934 |
| Individual 31 | 38.3 | 37.77 | 205.5119518 |
| Individual 32 | 36.4 | 36.33 | 227.189745 |
| Individual 33 | 36.5 | 36.55 | 216.1628906 |
| Individual 34 | 36.9 | 36.83 | 232.577867 |
| Individual 35 | 36.8 | 36.33 | 182.5811069 |
| Individual 36 | 36.9 | 36.33 | 242.3516697 |
| Individual 37 | 37 | 36.44 | 207.3915292 |
| Individual 38 | 36.7 | 37.33 | 238.5925148 |
| Individual 39 | 37 | 36 | 145.866694 |
| Individual 40 | 36.9 | 36.72 | 211.9025151 |
| Individual 41 | 36.7 | 36.44 | 172.3060835 |
| Individual 42 | 36.8 | 35.77 | 197.7430316 |
| Individual 43 | 37 | 35.44 | 229.6958483 |
| Individual 44 | 37.4 | 36.55 | 241.2239233 |
| Individual 45 | 36.8 | 37.44 | 109.5281967 |
| Individual 46 | 36.6 | 36.33 | 187.8439237 |
| Individual 47 | 36.7 | 37.05 | 119.4273046 |
| Individual 48 | 35.9 | 36.83 | 215.2857545 |
| Individual 49 | 36.5 | 36.77 | 169.1734544 |
| Individual 50 | 36.4 | 36.66 | 140.6038772 |

Endocrinological Testing:

Principle of the Test: The Cortisol test kit from Cal biotech, Inc. is a solid phase competitive ELISA. The specimens performing Cortisol-HRP amalgamation and Hydrocortisone acetate-biotin mixture are put into the streptavidin-coated wells.

Reagents Preparations:

Hydrocortisone-enzyme Conjugate Solution: In an appropriate container, Watery the hydrocortisone enzyme compound 1:21 with assessment dilutants.

Fender for washing: Add the bottle's ranges (25ml, 5X) to 119 ml of filtered or purified water to make a 1X Wash fender. Stock at room temperature at 25 degrees Celsius.

Figure 2: Thermal Infrared Gun and Digital Thermometer for the measurement of body temperature



Assay Method: Allow indicators to cool to room temperature before using. Before using, gently combine all reagents.

1. Fill the holder with the desired number of coated strips.
2. Pipette a total of 25l of normal hydrocortisone, control, and sufferer serum.
3. Fill each well with 50 l of Biotin reagent.
4. Fill each well with 100 ml of Cortisol Enzyme Conjugate.
5. Mix thoroughly for ten counts.
6. Hide at room temperature 25 (°C) for one hour.
7. Drain all mixture from the wells. 300 l of 1X wash buffer washed three times through the wells. Using absorbent paper towels blot.
8. Fill each well with 100 l of TMB substrate.
9. Allow for a 15-minute incubation period at ambient temperature (20-25°C).
10. Fill all wells with 50l of ending solution and mix the solution, greatly shake the plate.
11. Within 20 minutes after mixing the ending solution, examine the absorbance on an ELISA device at 450 nm.

Calculations of Consequence: Following is how the standard curve is made:

1. Check each standard vial's Cortisol standard value.
2. On a piece of linear graph paper, make the absorbance of hydrocortisone normal (upright central line) against hydrocortisone normal concentrations (horizontal central line) to create the standard curve. Draw the best arc possible between the spots.
3. Study the absorbance from the curve for the controls and every new sample. For each control or unknown sample, make a note of the value.

Example of normal Concentration:

| | Observed Data 450 nm | Concentration (ng/ml). |
|----------|----------------------|------------------------|
| Standard | 2.77 | 0 |
| Standard | 1.42 | 20 |
| Standard | 0.79 | 50 |
| Standard | 0.43 | 100 |
| Standard | 0.22 | 200 |
| Standard | 0.12 | 500 |

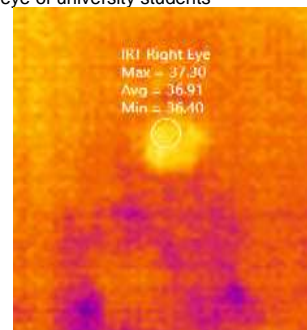
RESULTS

Correlation coefficient: A spearman correlation was observed between cortisol concentration with different methods of temperature measurements as digital thermometer temperature, temperature of infrared gun and infrared thermometer.

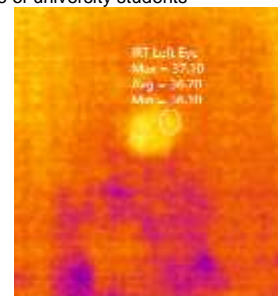
Infrared Thermal Temperature of Right Eye: Insignificant negative correlation was observed between cortisol concentrations (ng/ml) with body temperature of IRT of right eye-maximum (cc = -0.100; p = 0.490 respectively). Insignificant negative correlation was observed between cortisol concentrations (ng/ml) with body temperature of IRT of right eye-minimum (cc = -0.046; p = 0.750 respectively). Insignificant negative correlation was observed between cortisol concentrations (ng/ml) with body temperature of IRT of right eye-average (cc = -0.085; p = 0.558 respectively) (index table 1).

Infrared Thermal Temperature of Left Eye: Insignificant negative correlation was observed between cortisol concentrations (ng/ml) with body temperature of IRT of left eye-maximum (cc = -0.099; p = 0.495 respectively). Insignificant negative correlation was observed between cortisol concentration (ng/ml) with body temperature of IRT of left eye-minimum (cc = -0.111; p = 0.442 respectively). Insignificant negative correlation was observed between cortisol concentration (ng/ml) with body temperature of IRT of left eye-average (cc = -0.031; p = 0.833 respectively) (index table 2).

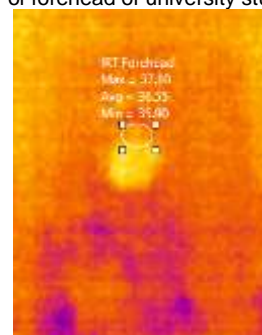
IRT image of right eye of university students



IRT image of left eye of university students



Example of an IRT of forehead of university students.

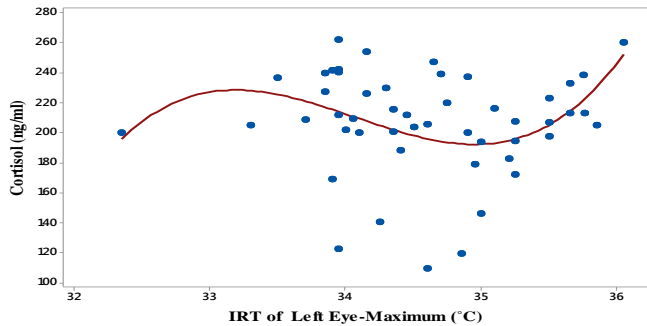


Infrared Thermal Temperature of Forehead: Insignificant negative correlation was observed between cortisol concentrations (ng/ml) with body temperature of IRT of forehead-maximum (cc = -0.124; p = 0.413 respectively). Insignificant negative correlation was observed between cortisol concentration (ng/ml) with body temperature of IRT of forehead-minimum (cc = -0.034; p = 0.822 respectively). Insignificant negative correlation was observed between cortisol concentration (ng/ml) with body temperature of IRT of forehead-average (cc = -0.090; p = 0.551 respectively) (index Table 3).

Table 1: Spearman correlation of infrared thermal temperature of eye and forehead with Cortisol in University Students.

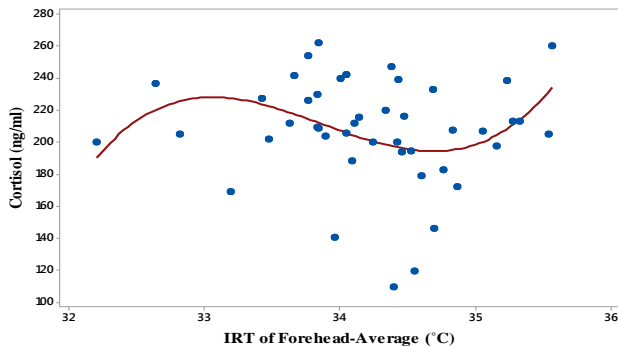
| Infrared thermal temperature | Physiological parameter | Correlation coefficient | P value |
|---|-------------------------|-------------------------|---------|
| Table 1: Infrared Thermal Temperature of Right Eye | | | |
| Right eye (maximum) | Cortisol | -0.100 | 0.490 |
| Right eye (minimum) | Cortisol | -0.046 | 0.750 |
| Right eye (average) | Cortisol | -0.085 | 0.558 |
| Table 2: Infrared Thermal Temperature of Left Eye | | | |
| Left eye (maximum) | Cortisol | -0.099 | 0.495 |
| Left eye (minimum) | Cortisol | -0.111 | 0.442 |
| Left eye (average) | Cortisol | -0.031 | 0.833 |
| Table 3: Infrared Thermal Temperature of Forehead | | | |
| Forehead (maximum) | Cortisol | -0.124 | 0.413 |
| Forehead (minimum) | Cortisol | -0.034 | 0.822 |
| Forehead (average) | Cortisol | -0.090 | 0.551 |

Figure 1: Cubic relationship of cortisol concentration (ng/ml) = - 524684 + 46279 of maximum left eye - 1359 of maximum left eye² + 13.30 of maximum left eye³ {r²adj = 7.9%; F- value =6.03; P-value = 0.018}with Infrared thermal temperature (IRT) of left eye (maximum) in university students.



The cubic relationship of cortisol concentration (ng/ml) measured by IRT of forehead-average (°C) was significantly (p = 0.035) correlated with the body temperature of individuals (Figure 2).

Figure 2: Cubic relationship of cortisol concentration (ng/ml) = - 604253 + 53597 of average of forehead - 1583 of average of forehead² + 15.58 of average of forehead³ {r²adj = 5.2%; F- value =4.77; P-value = 0.035}with Infrared thermal temperature (IRT) of forehead (average) in university students.



Temperature of Infrared Gun and Digital thermometer: Insignificant positive correlation was observed between cortisol

concentrations (ng/ml) and thermal infrared gun (cc = 0.029; p = 0.844). Insignificant negative correlation was observed between cortisol concentrations (ng/ml) and digital thermometer (cc = -0.010; p = 0.946 respectively) (Index Table 4).

Regression: In regression model cortisol concentration (ng/ml) was significantly correlated with temperature measured by different techniques as temperature of infrared gun, digital thermometer temperature and (IRT). The cubic relationship of cortisol concentration (ng/ml) was significantly (p= 0.018) correlated with the body temperature of individuals measured by IRT of left eye-maximum (°C). (Figure 1).

The cubic relationship of cortisol concentration (ng/ml) was significantly (p = 0.056) correlated with body temperature of individuals taken through digital thermometer (°C) from facial region (Figure 3).

Figure 3: Cubic relationship of cortisol concentration (ng/ml) = 2392261 - 196253 digital thermometer temperature + 5366 digital thermometer temperature² - 48.88 digital thermometer temperature³ {r²adj = 10.6%; F- value =4.16; P-value = 0.056} with digital thermometer temperature (°C) in university students.

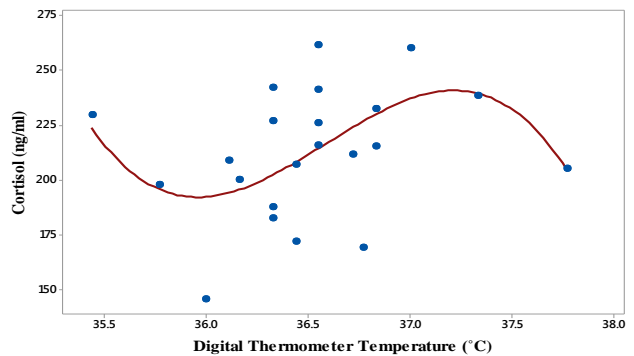
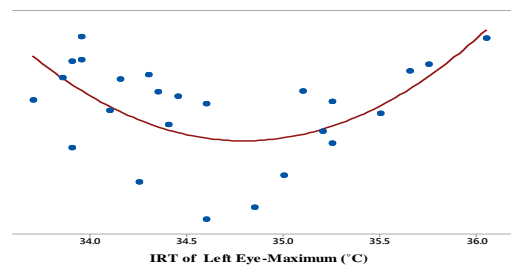
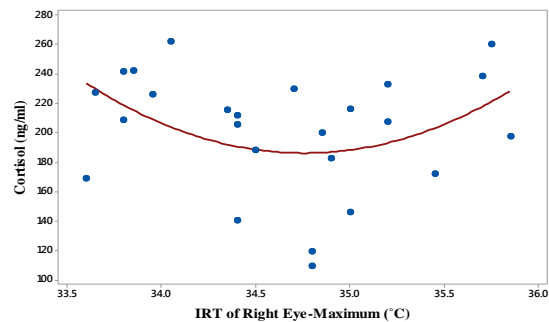


Figure 4: Quadratic relationship of cortisol concentration (ng/ml) = 43056 - 2467 of maximum right eye + 35.48 of maximum right eye²{r²adj = 8.2%; F- value =3.96; P-value = 0.059} and quadratic relationship of cortisol concentration (ng/ml) = 71466 - 4098of maximum left eye + 58.8 of maximum left eye² {r²adj = 29.5%; F- value =15.05; P-value = 0.002} with Infrared thermal temperature (IRT) of right eye (maximum) and of left eye (maximum) in university students.



The quadratic relationship of cortisol concentration (ng/ml) observed by IRT of right eye-maximum (p= 0.059) and IRT of left

eye-maximum ($p= 0.002$) was significantly correlated with body temperature of individuals (Figure 4).

The cubic relationship of cortisol concentration (ng/ml) was significantly correlated with body temperature of individuals measured by IRT of forehead-minimum ($p= 0.012$) and IRT of forehead-average ($p= 0.019$) (Figure 5).

The cubic relationship of cortisol concentration (ng/ml) observed by IRT of eye-maximum ($p= 0.019$) and IRT of eye-average ($p= 0.050$) was significantly correlated with body temperature of individuals (Figure 6).

Figure 5: Cubic relationship of cortisol concentration (ng/ml) = $- 1577271 + 140539$ of minimum forehead - 4172 of minimum forehead² + 41.28 of minimum forehead³ [$r^2_{adj} = 23.0\%$; F- value = 7.80 ; P-value = 0.012] and cubic relationship of cortisol concentration (ng/ml) = $- 2583324 + 226738$ of average of forehead - 6631 of average of forehead² + 64.63 of average of forehead³ [$r^2_{adj} = 20.9\%$; F- value = 6.49 ; P-value = 0.019] with Infrared thermal temperature (IRT) of forehead (minimum) and of forehead (average) in university students.

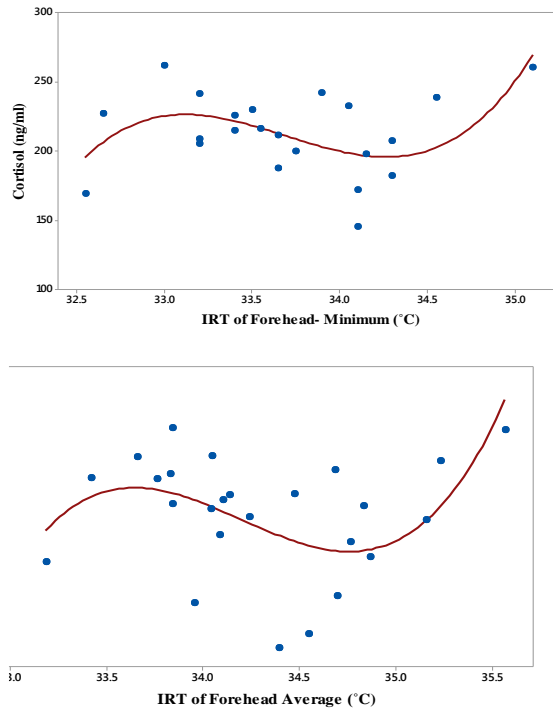


Figure 6: Cubic relationship of cortisol concentration (ng/ml) = $- 1381942 + 119896$ maximum of eye - 3466 maximum of eye² + 33.38 maximum of eye³ [$r^2_{adj} = 19.3\%$; F- value = 6.75 ; P-value = 0.019] and cubic relationship of cortisol concentration (ng/ml) = $- 1003756 + 88393$ Average of eye - 2593 average of eye² + 25.35 average of eye³ [$r^2_{adj} = 7.4\%$; F- value = 4.36 ; P-value = 0.050] with Infrared thermal temperature (IRT) of eye (maximum) and eye (average) in university students.

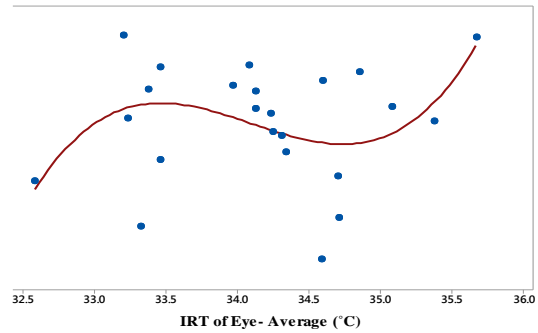
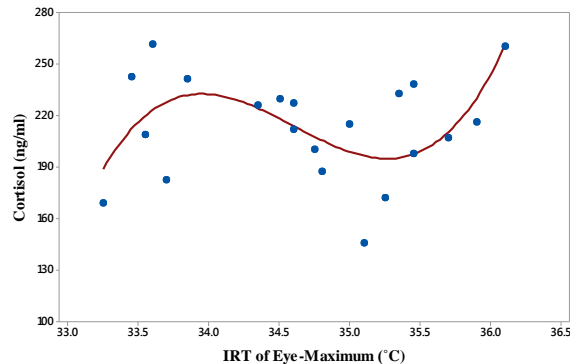
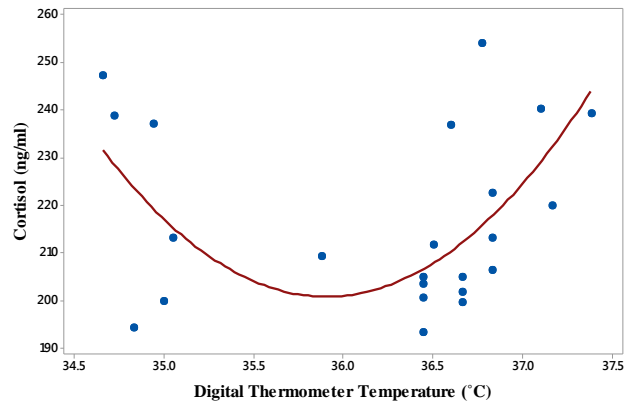


Figure 7: Quadratic relationship of cortisol concentration (ng/ml) = $25788 - 1425$ digital thermometer temperature + 19.85 digital thermometer temperature² [$r^2_{adj} = 25.5\%$; F- value = 9.50 ; P-value = 0.006] with digital thermometer temperature (°C) in university students.



DISCUSSION

In our study trial total 65 people were included from which 50 samples were collected (25 were females and 25 were males). The main objective of this study was to examine the relationship between body temperature and serum cortisol concentration in university students. A significant negative correlation was observed in cortisol concentration with IRT of eye and forehead, thermal infrared gun and digital thermometer. We observe that cortisol concentration (ng/ml) was significantly ($p = 0.018$) correlated with IRT of left eye (°C). In the past, a possible role of hydrocortisone for the activation of fatty tissues in living organisms has been studied. Our research states that hydrocortisone is significantly positively correlated with supraclavicular tissues of adult humans¹³, emphasizes the impact of hydrocortisone on human fatty tissues as an attractive route for coming research.

However, we discovered significant and optimistic correlations among the differences amid serum hydrocortisone or lactogenic hormone with human core temperature. Digital thermometers can observe human body temperature very quickly and with accurate reading. Calculated temple temperature with the help of three handholding infrared thermometers for 1000 fit individuals. Temperature measured by two kinds of infrared thermometers was (33.3°C) and (1.18°C). On the other hand, the temperature measured by three kinds of thermometers was unreliable. According to this research, if the temple temperature exceeds (35.5°C) indicates fever⁶.

The standard distinction between the infrared thermal temperature of the ear and a non-invasive thermometer that measures the temperature of forehead skin was lower than (1°C)¹⁷⁻¹⁸. Infrared thermography uses human skin to measure temple temperature, not the human muscle¹⁹.

The limitations in the working of IRT are that it is influenced by measurement procedure and ecological ingredient like solar power and air stream.

CONCLUSION

Infrared thermography is an imaging modality that uses thermal radiation to detect the body temperature. In the new Covid days, a non-contact infrared gun is used to measure individual's temperature from a distance. Cortisol is a protein and cortisol concentrations are non-significantly correlated with IRT of forehead and eyes.

Authorship and contribution declaration: Each author of this article fulfilled following Criteria of Authorship:

1. Conception and design of or acquisition of data or analysis and interpretation of data.
2. Drafting the manuscript or revising it critically for important intellectual content.
3. Final approval of the version for publication.

All authors agree to be responsible for all aspects of their research work.

Conflict of interest: None

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