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Cortisol detection methods for stress monitoring in connected health

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ABSTRACT

Everyday responsibilities and lifestyle issues are the main cause of physical and psychological stress, which deteriorates the individual's health. Prolonged exposure to stress triggers the adrenocorticotrophic hormonal (ACTH) system and causes the release of cortisol hormones from the adrenal cortex. Many other biomarkers are affected by stress, but cortisol is considered the most vital and potentially clinically useful biomarker for stress estimation and monitoring. Accurate and timely detection of increased cortisol levels might improve the diagnosis, treatment, and prevention of stress-related diseases such as anxiety disorders, metabolic dysregulation, and cardiovascular diseases. Unfortunately, most of the cortisol assessments are currently performed only in laboratories and there is no point-of-care solution for ambulatory/real-time cortisol assessment. This review aims to provide an overview of the most promising techniques, currently used for cortisol detection and the challenges associated with them. The review also provides a feasibility report about measuring cortisol levels in different bio-fluids (for example, urine), a correlation of perceived stress with cortisol levels, and methods/devices used in the laboratory as well as in the ambulatory environment for cortisol detection. The overall conclusion suggests that significant research efforts and investments are required for the development of an accurate, rapid, and repeatable cortisol measuring device that can be used for connected health applications.

Introduction

Stress is a physical, emotional, and physiological response of the body to an internal or external stimulus, categorizing it as one of the major threats to mental health [1]. The increasing psychological stress levels due to an altered lifestyle, globalization, and competition are of serious concern, causing life-threatening diseases such as depression, heart attacks and stroke [2]. Thus, the accurate and precise detection of physiological as well as psychological stress is gaining the attention of researchers and investigators for personalized health monitoring and diagnostics [3]. Current stress diagnostic approaches include the measurement of stress effects, stress exposure, self-reporting questionnaires and assessment of different biomarkers [4]. Among these, a biomarker-based stress assessment system is considered unequivocal for an effective diagnostic approach [5].

In recent years, wearable stress monitoring devices have been developed to relate stress with abnormalities in the environment and to gain vital information for real-time diagnosis and treatment. Since the development of the Trier Social Stress Task (TSST) [6] in the year 1993, our knowledge about the neuro-endocrine processes associated with physiological and/or psychological stress has increased significantly. Research with laboratory-based stress induction tests like TSST aims to understand the impact of stress on hormonal stress responses of a human body under controlled conditions [7]. The TSST emphasizes the critical role of cortisol blood levels in the detection of stress [8]. Several studies have performed experiments with students, police officers, nurses, and athletes to link the levels of cortisol with stress and have classified cortisol as the most prominent biomarker for stress detection [9–16].

Cortisol ($C_{21}H_{30}O_5$), is a steroid hormone with a molecular weight of 362.46 g/mol. Cortisol is a well-known biomarker of psychological and physiological stress [17,18], see Fig. 1. The level of cortisol plays an important part in regulating blood pressure, carbohydrate metabolism and glucose levels. It also contributes to the homeostasis of cardiovascular, renal, immune, endocrine and skeletal systems [9,10,19]. Abnormally increased levels of cortisol interfere with blood amino acid and fatty acid levels, resulting in depression of the immune system and inflammation. Severely increased levels of cortisol contribute to the development of symptoms of obesity, bone fragility, and fatigue [20], while decreased levels of cortisol lead to Addison's disease manifested by arterial hypotension, weight loss and darkened scars/skinfolds [21]. The most

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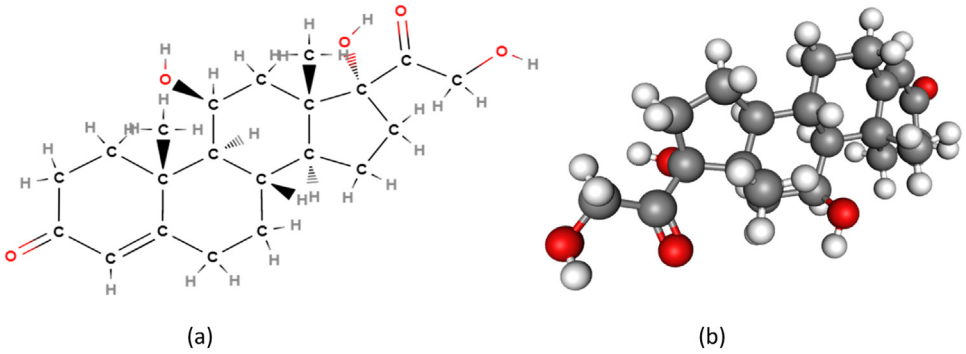

Fig. 1. Molecular and 3D structure of Cortisol ($C_{21}H_{30}O_5$). In (a) C is for Carbon, H is for Hydrogen and O is for Oxygen molecule. In (b) Black shows Carbon, grey shows Hydrogen and Red shows Oxygen molecule. (Generated using: https://molview.org/?cid=5754).

Table 1
Comparison with recent review papers with the proposed review article.

Refs	Correlation of cortisol with stress	Feasibility (invasive/ non-invasive)	Techniques Ambulatory settings	Techniques Laboratory settings	Future Stress management	cortisol measuring methods reviewed
[3]	√	✗	√	√	✗	5
[24]	√	✗	√	✗	√	3
[28]	✗	✗	✗	√	√	4
[29]	√	✗	√	✗	√	6
[30]	✗	✗	√	✗	✗	5
[31]	✗	✗	✗	√	√	6
Proposed	√	√	√	√	√	**6**

dominating effects of cortisol are indicative of emotional or psychological stress and that is why cortisol is also called the 'stress hormone' [22].

Currently, in the clinic, total cortisol (i.e., the sum of protein-bound and free fractions) is measured. The free cortisol is the only biologically active fraction and is liable for all cortisol-related effects in the body and could be found in blood (serum and plasma), saliva, urine and other biological fluids [23]. Although the reference values of cortisol levels that could be translated to an individual's physical or psychological stress are yet to be determined, it would be of value to develop an accurate diagnostic system that allows repeated measurements of free cortisol [24]. The currently available methods for the determination of free cortisol levels are mostly laboratory-based. These strategies require large samples, are time-consuming, laborious as well as expensive and are not suitable for point-of-care diagnostics [25–27]. Furthermore, these current set-ups only provide a glimpse at cortisol levels within the sample submitted to the laboratory and do not provide a realistic representation of its variations. There is also an influence of the time of the day on the cortisol levels within the body. Thus, one tends to rely upon 24 h urine analysis of cortisol metabolites, instead of cortisol sampling. Recent advances in technology have shown prominent results towards the development of such systems.

To review the existing technologies developed to self-test cortisol levels, this article highlights the recent efforts made to develop strategies for the detection of cortisol in laboratory and in point-of-care/ambulatory (where healthcare is provided close to the patient, for example in home) settings. Moreover, this review discusses the correlation of cortisol levels with induced stress, the comparison of different cortisol detection techniques and provides future directions regarding the development of a real-time cortisol measuring stress monitoring device. In the present literature, most of the review papers are specific to either cortisol detection in the laboratory or cortisol detection in point-of-care/ambulatory settings. In comparison with previously published reviews, shown in Table 1, this review paper will answer the following questions (that no other single review covers):

- The correlation between induced stress and cortisol levels.
- The feasibility of cortisol detection using invasive and non-invasive methods.
- The devices/techniques used for detecting cortisol in laboratory settings.
- The devices/techniques used for detecting cortisol in point-of-care/ambulatory (near patient/outside hospital) settings.
- The current research status regarding the development of a real-time cortisol measuring stress monitoring device and the future direction.

The rest of the review article is organized as follows: Section 2 describes the search terms and inclusion criteria; Section 3 provides a brief feasibility report of different cortisol sampling sources, Section 4 reviews the existing literature to determine the relationship of cortisol levels with induced stress and provides correlation analysis. Sections 5 discusses the application of cortisol detection in clinical research. Section 6 and 7 discuss the literature related to cortisol sensing mechanisms in the laboratory and point-of-care (ambulatory) settings. Lastly, discussion, conclusion and future directions are presented in Section 8.

Search methodology

The method and design used for the compilation of this review comply with the PRISMA guidelines [32] which is the preferred reporting process for meta-analyses and systematic reviews. The search engines used to select the studies were PubMed, Google Scholar, IEEE Xplore, Elsevier, ScienceDirect and Wiley online library. The search terms were the combination of three general keywords (cortisol, stress, and detection) with a maximum of three specific keywords (correlation, feasibility, invasive/non-invasive, point-of-care/ambulatory settings, real-time, laboratory settings, monitoring devices, blood/plasma/serum, urine, hair, sweat, saliva, and tears). Initially, 905 studies were retrieved using various combinations of the abovementioned search terms. The result of the PRISMA searches and the total number of selected studies are presented in Fig. 2.

Feasibility of different sources for cortisol sampling

The cortisol hormones are secreted by adrenal glands located above our kidneys. It is the end-product of an important component of the human's body adaptive system called the hypothalamic-pituitary-adrenal (HPA) axis. HPA regulates the physiological processes of the body under different environmental factors [3]. In response to triggers, the hypothalamus in the brain releases a corticotrophin-releasing hormone

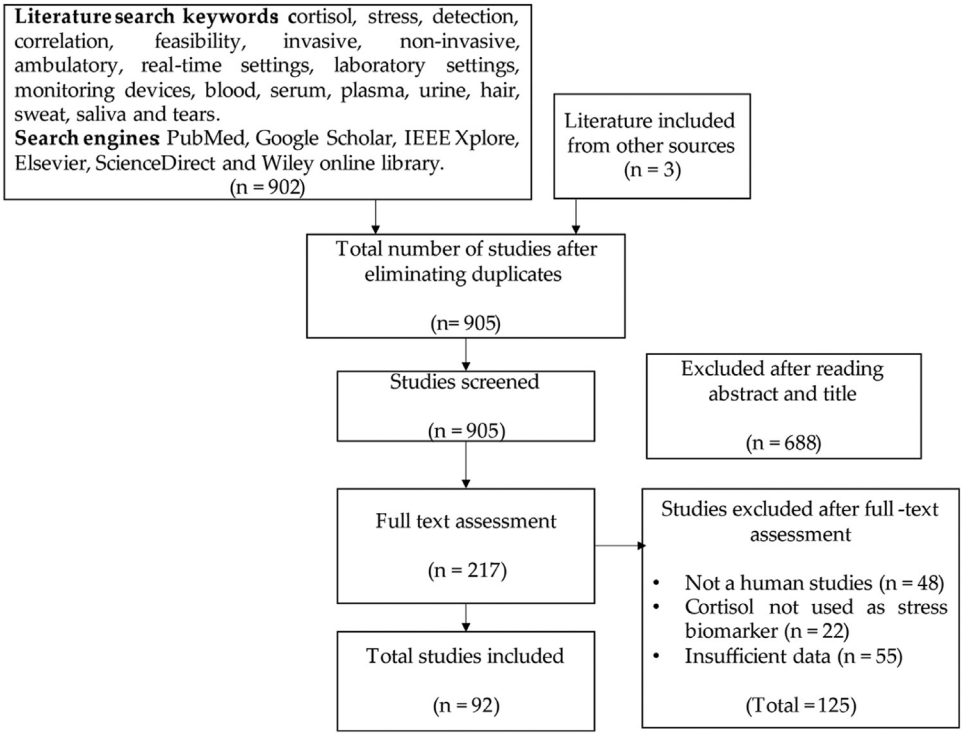

Fig. 2. Adopted PRISMA guidelines and retrieved papers in this review.

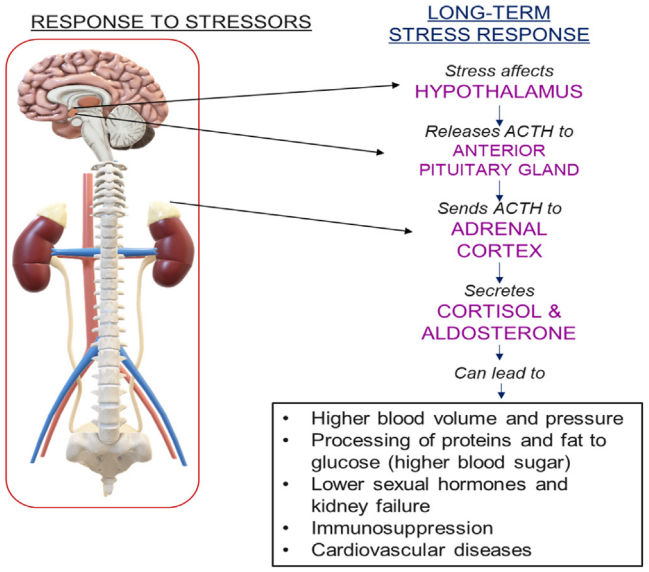

Fig. 3. Illustration of cortisol production as a response to long-term stress.

(CRH) acting at the pituitary glands. The pituitary glands are release adrenocorticotrophic hormones (ACTH) into the blood that travels to the adrenal cortex. The adrenal cortex responds to ACTH by increasing the production of cortisol, which then participates in modulating a number of physiological processes. The secreted cortisol steers its way to the circulatory system and can be found in several biological fluids in detectable quantities. Fig. 3 illustrates the process of the body's response to a stressor (a stimulus that induces stress). This section assesses the feasibility, advantages, and disadvantages of sampling different sources of cortisol based on literature data.

Salivary cortisol

Over the recent years, salivary cortisol detection has gained considerable attention for the development of stress monitoring systems. This is mainly due to the advantages associated with saliva as a convenient bio-fluid. The first and most important fact is the well-documented literature that shows a strong correlation between blood and salivary cortisol concentrations [33]. The other important advantage of salivary cortisol is that it is entirely in a free state. Harvesting salivary cortisol samples is also a completely non-invasive and painless process. A standard operating procedure has been established for saliva collection, which leads to reduced variability of measurements. Along with the above-mentioned advantages, there are some drawbacks as well. The nominal values of salivary cortisol during the diurnal cycle vary from 0.5 μg/dL to 0.05 μg/dL [34]. Thus, a highly sensitive assay with the ability to detect low ranges of cortisol is required. Also, the salivary cortisol is highly unstable at room temperature causing problems of storage throughout the on-site sampling and processing period. In the mornings, the mean salivary cortisol concentration is in the range of 3.6 nmol/L to 8.3 nmol/L while at late nights the concentration drops to 2.95 nmol/L to 2.1 nmol/L [35].

Hair cortisol

Human hair grows at a predictable rate of almost 1 cm per month. The cortisol hormone is known to be found in the shaft of the hair. Also, the proximal 1 cm segment of hair (closer to the scalp) approximates the previous month's cortisol production [36]. The hair cortisol is hypothesized to reflect the free cortisol fraction rather than total cortisol concentration in the blood plasma and serum. The hair cortisol reference values vary from 1.7 to 153.2 pg/mL (1.7×10^{-6} μg/mL to 0.0001532 μg/mL) [37]. The hair cortisol measurement is a non-invasive way of obtaining a biological sample. Hair cortisol levels are representative of long-term exposure to stress. Because the data are "collected" over several months, determining the association of hair cortisol with stress will require careful correlation with frequently obtained clinical data, not available so far in the literature.

Urine cortisol

Cortisol levels measured in urine are referred to as 24 h urinary free cortisol (UFC). Only free and active cortisol is present in the human

urine, qualifying urine samples as a relevant bio-fluid for cortisol detection. The normal range of UFC levels is 36 μg/24 h to 137 μg/24 h [38]. Although the collection of 24 h urine for UFC test is a non-invasive and painless method, it also poses some issues regarding convenience and reliability. Since the sample is collected over 24 h, the patient must carry a special urine collecting container all day and must be relatively confined to a given location for 24 h. Also, the container needs to be stored in a refrigerator from the time of collection to its delivery to the laboratory. Moreover, with the entire volume (24 h urine), creatinine is also needed to be measured to verify that the collection is complete. Factors such as pregnancy and medication (such as ketoconazole, adrenalux and metyrapone) can alter the concentration of cortisol in the urine sample. The requirement for 24 h collection of the urine renders the UFC measurement poorly suited for real-time cortisol detection at point-of-care settings.

Blood (serum and plasma) cortisol

The assays used to measure cortisol levels in the blood measure the sum of bound and free cortisol, the latter being the active form. The Cortisol Free Index (CFI) is calculated using Coolen's equations [23]. Under non-stress conditions, 10 to 15% of the blood cortisol is bound to serum albumin while 80 to 90% is bound to corticosteroid-binding globulin (CBG). The remaining 5–10% is in a biologically active state and participates in cortisol-initiated effects [39]. The normal range of blood cortisol varies from morning to evening. The nominal value of total blood cortisol level is 0.05 μg/mL to 0.25 μg/mL (25 μg/dL at 9 am versus 2 μg/dL at midnight) [3]. There are several drawbacks of sampling cortisol from blood, making it a suboptimal sampling site. The main drawbacks are listed below:

- Collecting blood samples requires trained staff and sterilized equipment with the potential effect of stress, fear, and pain on the cortisol levels.
- Cortisol molecules are unstable at room temperature and the plasma requires special handling and storage environments.
- Sampling blood needs vein puncturing which can be painful and is often perceived as stressful. As a result of pain, cortisol levels will immediately increase.
- Although the typical time of cortisol spiking after a stressful event is 10 to 15 min in humans, venipuncture and the apprehension that is associated with the need for blood sampling can initiate stress-induced cortisol spiking.
- Time of the day needs to be considered for interpretation of the result and a standardized sampling technique in a quiet environment is desirable.
- The cost of equipment, staff, handling, and storage makes blood cortisol sampling a shunned option.

Interstitial fluid cortisol

Interstitial fluid (ISF) is the extracellular fluid that envelops cells in the tissues. ISF is similar to blood plasma in composition. Generally, small to moderate-sized molecules (0.5–5 nm), such as ethanol, glucose, and cortisol (362.46 g/mol) are found in ISF in the same ratio as in blood plasma. The use of microneedles to obtain ISF in a painless, minimally invasive manner, has been successfully developed [40–43]. For cortisol detection, ISF needs to be harvested at a very slow rate of 10 μL/h which limits its applicability in the point-of-care/ambulatory setting. The concern regarding biodegradation and biocompatibility of microneedles, risk of infection, continuous extraction of body fluid and other sterilization issues need to be addressed carefully for successful implementation of the ISF cortisol detection approach.

Sweat cortisol

Sweat cortisol measurements have found reference values of cortisol concentration in the sweat, ranging from 8.16 ng/mL to 141.7 ng/mL (0.00816 μg/mL to 0.1417 μg/mL) [44]. For the sweat collection, sweat patches are frequently used which is an effective non-invasive method to do so. However, due to very limited knowledge of cortisol correlation in sweat and several other factors such as humidity, temperature, physical activity, geographic condition, and individual genetic factors, there are inherent limitations to the development of a reliable and repeatable sweat sampling device for cortisol detection.

Perceived stress and cortisol levels: correlation analysis

To determine the correlation between stress and cortisol levels there is a vast literature available on non-invasive methods of cortisol detection, such as cortisol in saliva, hair, urine, sweat and fingernails while limited literature is available on invasive methods of cortisol detection (such as, in plasma and serum). The following literature has been reviewed to determine the true relationship of stress-induced cortisol levels in different biospecimens. Fig. 4 illustrates the number of reviewed literature reporting positive, negative and no significant correlation of cortisol level, measured via different technique, with induced stress.

Salivary cortisol

Siddiqui et al. [45] recruited 125 subjects with newly diagnosed diabetes mellitus and 125 subjects with normal glucose. The radioimmunoassay method was used to determine the cortisol levels in the saliva of the participants. The authors found high cortisol levels in subjects with newly detected diabetes (3.62 ± 3.53 nmol/L, $P < 0.001$) as compared to salivary cortisol subjects with normal glucose (2.25 ± 1.67 nmol/L, $P < 0.001$). Thus, there is a positive correlation between stress and cortisol levels.

Similarly, Grossi et al. [46] determined the level of salivary cortisol (in the morning) of 64 patients with either high, low, or moderate burnout symptoms. The authors also used the radioimmunoassay method to determine cortisol levels. The authors reported increased (mean) levels of cortisol in all female patients. The mean increase in cortisol levels for a low burnt-out woman was 4.14 ± 4.69 nmol/L, medium burnt-out was 4.75 ± 4.68 nmol/L and high burnt-out was 2.98 ± 6.39 nmol/L. However, cortisol levels were only elevated in male patients with moderate burnout, and not in other male patients or healthy subjects.

Powell et al. [47] studied 20 divorced or widows (defined as stress group) and a group of 20 non-stressed women engaged in a stable marriage. The authors collected evening cortisol levels in both groups using radioimmunoassay with a time-resolved fluorometric detection technique. Increased levels of evening cortisol were detected in the group of stressed women as compared to non-stress women (mixed-effects model: effect = 0.44; standard error = 0.14, $p = 0.003$), thus establishing a positive correlation between cortisol levels and stress.

Carlesso et al. [48] investigated the relationship between salivary cortisol and osteoarthritis (OA) pain using cotton Salivettes (Salivette kit). They recruited 31 women with hip, spine, or knee osteoarthritis pain and monitored their salivary cortisol levels at a different time of a day for 7 days. The authors concluded that there is a positive association of cortisol production with stressful pain as the greater the pain, the greater the cortisol levels in the saliva. The reported unstandardized β co-efficient was 0.083 and the p-value was 0.009 which showed an 8.7% increase in cortisol levels in women with OA.

Schmalbach et al. [49] performed an experimental study on 52 subjects (26 anorexia nervosa patients and 26 healthy control subjects). The author monitored the salivary cortisol levels under two conditions: firstly, at rest, next during stress intervention using the Trier Social Stress Test (TSST). The cortisol levels were measured using a Salivette

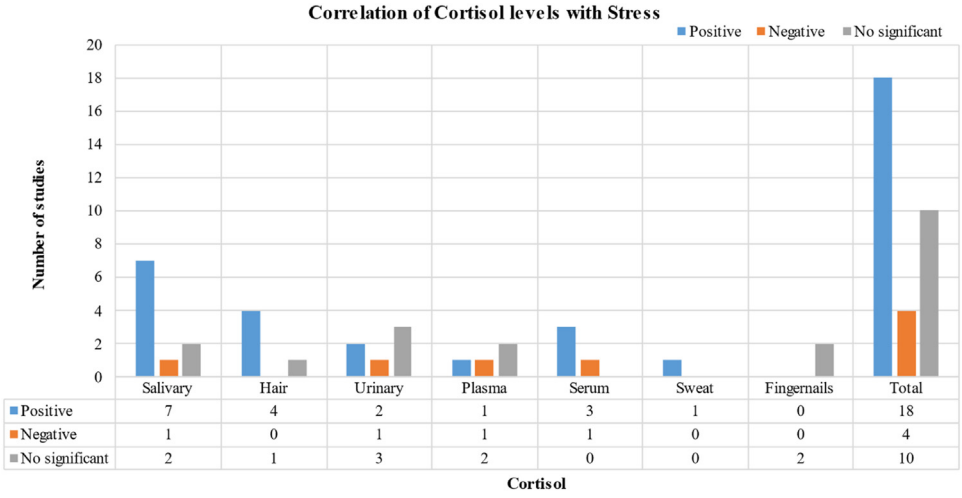

Fig. 4. the number of reviewed literature reporting positive, negative and no significant correlation of cortisol level, measured via different technique, with induced stress.

kit. The authors concluded that in both groups, the overall cortisol levels were elevated after the stress was induced. Surprisingly, there was no significant difference in the cortisol levels between the two groups i.e., patient vs healthy control ($p = 0.410$).

Balters et al. [50] assessed the correlation between salivary cortisol and induced stress. The authors experimented in a controlled environment and induced three levels of stress intensity (baseline, low and high). A total of 12 ship captains were recruited and were asked to complete a self-reporting questionnaire to determine their stress levels. A Salivette Code Blue was used for saliva collection. The authors concluded that there is a significant increase in the salivary cortisol levels during the high-stress period (with an expected 20 and 40 min delayed peak response, depending on stimulus stressor). The determined chi-squared correlation co-efficient was 19.5 (p-value <0.001).

Ethridge et al. [51] examined the association of hypothalamic-pituitary-adrenal (HPA) axis stress response with salivary cortisol. They recruited 112 men and randomly divided them into stress (experimental) and control groups. The author measured the salivary cortisol levels using a Salivette collection device and used computerized arithmetic challenge along with a social evaluation to induce stress. Maximum likelihood estimation was used to determine the correlation of cortisol levels with stress. The authors observed reduced cortisol output under the stress conditions and concluded that there is a negative correlation (co-efficient =−2.17, p-value = 0.04) between cortisol levels and induced stress.

Mizuhata et al. [52] monitored the effect of breastfeeding on stress and depression using salivary cortisol. The authors examined 79 women and used the perceived stress scale (PSS-10), breastfeeding self-efficacy scale, and Edinburgh postnatal depression scale to determine the stress levels. The ELISA kit was used to collect the saliva samples from the participants. The authors concluded that breastfeeding reduces stress levels and a significant decrease in salivary cortisol levels was noted after the experiment ($\beta = -0.260$, p-value < 0.05).

Jansakova et al. [53] aimed to assess the effect of a stressed day on the flow of salivary cortisol, as compared to an ordinary day. The authors collected saliva samples of 42 children using an ELISA kit. Stress was induced by taking test questions that evaluated their skills in mathematics, mother tongue, foreign language, and knowledge about natural sciences. The authors observed no significant correlation between the salivary cortisol and stress/non-stress condition.

Metz et al. [54] compared the HPA axis and sympathetic nervous system (SNS) responses of posttraumatic stress disorder (PTSD) patients and healthy individuals under psychosocial stress and no-stress condition. The authors recruited 32 healthy women and 21 female patients. Trier social stress test (TSST) and placebo-TSST were used to induced stress during the experiment. Saliva samples were collected using a Salivette collection device. The authors found that there is a positive correlation (p-value = 0.01, Pearson's Chi^2 co-efficient; η^2 =0.12) between the perceived stress and salivary cortisol in patients with PTSD.

Hair cortisol

Pompon et al. [55] conducted a study with 57 participants to determine the relationship of depression/anxiety and cortisol levels with perceived stress. They used hairs of 1 cm in length to detect the cortisol levels with Salimetrics High-Sensitivity Cortisol EIA kit. The authors used Pearson product-moment correlational analysis and concluded that there is no significant correlation between chronic stress and cortisol levels if the participant is less depressed or has a low level of anxiety ($r = 0.47$, p-value = 0.14). They also concluded that there is a significant association ($r = 0.56$, p-value = 0.04) of high levels of cortisol in subjects with moderate or high levels of depression in relation to excess stress.

Vliegenthart et al. [56] conducted a study on 270 children and adolescents to determine the relationship between cortisol levels and socioeconomic status. Hair cortisol levels were determined using Liquid Chromatography-Tandem Mass Spectrometry (LCTMS). The authors concluded that there is an increase in cortisol levels in participants belonging to low socioeconomic stated families. A similar method of cortisol detection is also adopted by Chen et al. [57]. They recruited 87 children and 87 caregivers, who were obese and had some disability, to determine the correlation of cortisol with stress. The authors reported elevated hair cortisol concentration for stressed caregivers and concluded that there is a positive correlation between cortisol levels and stress in the caregiver as well as in children ($r = 0.23$, p-value = 0.036).

Henley et al. [58] conducted a study with a total of 55 participants divided into two groups; V1- first national volunteers and V2- non-first nation volunteers. The authors used an enzyme-linked immunoassay (ELISA) kit to determine the cortisol levels in the hairs of all the volunteers. They observed an increased concentration of hair cortisol in group V1 as compared to group V2 (189 ng/g versus 144 ng/g). The authors concluded as group V1 has greater stress levels, thus the elevated cortisol levels indicate its positive correlation with stress using Mann-Whitney U test (p-value<0.0001).

Bautista et al. [59] assessed whether hair cortisol levels were independently associated with hypertension. The authors measured hair cortisol levels and blood pressure of 75 participants using an ELISA kit. The perceived stress was measured using Depression Anxiety Stress Scales (DASS-21) [60]. The analysis showed that chronic stress results in increased systemic hair cortisol levels that eventually lead to the development of hypertension. Thus, there is a positive correlation ($r = 0.185$) between hair cortisol levels and perceived stress.

Urinary cortisol

Moch et al. [61] recruited 32 South African women (16 burnout and 16 non-burnt) and determined the relationship of urinary cortisol levels with stress using the radioimmunoassay technique. The author found out that there are reduced free cortisol in the burnt-out patients as compared to control subjects and there was no effect of stress management sessions in restoring the hypercortisolism state.

Cremeans-Smith et al. [62] investigated the urinary cortisol levels of 110 patients before and following knee osteoarthritis (OA) surgery. The author home delivered a polypropylene container to the participants to collect their urine samples. Low cortisol levels were detected before surgery and high cortisol levels after the surgery as patient-rated the post-surgery pain was more severe and were in stress.

Khoromi et al. [63] assessed 24 h urinary cortisol of 16 men with chronic pain and compared cortisol levels with 12 healthy men of the same body mass index and ages. The polypropylene container was used to collect the 24-hours urine samples and determine the cortisol levels. The authors did not notice any significant difference in mean as well as integrated concentrations of cortisol levels in patients compared to control subjects.

Butts et al. [64] determined the associations between urinary cortisol and psychological stress as well as in vitro fertilization (IVF) outcomes. They collected the urine samples from 28 males and 52 women using an ELISA kit. The authors do not identify any significant association of psychological stress and IVF outcomes with urinary cortisol levels.

Du et al. [65] conducted a cross-sectional on 60 bus drives and 44 supporting staff to validate the physiological stress against the questionnaire of occupational stress index (OSI) using urinary cortisol. The authors collected the 24-h urine samples and used the radioimmunoassay method to extract the levels of cortisol. They found no significant association between stress and urine cortisol.

Shimanoe et al. [66] assessed the association of cortisol-to-cortisone ratio with depression and perceived stress. The depression levels were measured using Zung self-Rating Depression Scale while perceived stress was measured using a self-reporting questionnaire. The authors analyzed urinary cortisol and cortisone levels of 6878 older adults using the Liquid-Chromatography Mass Spectrometry (LC-MS/MS) technique. They concluded that cortisol-to-cortisone ratio and cortisol levels were positively correlated to perceived stress. The authors reported the percentage change between stress and non-stress cortisol-to-cortisone ratio to be 2.33% with a p-value of 0.003 while cortisol levels to be 4.74% with a p-value of 0.001.

Plasma cortisol

Hall et al. [67] evaluated plasma cortisol levels in 102 patients undertaking elective arthroplasty for osteoarthritis (OA). The authors collected the blood samples from a cannula inserted in the forearm vein to determine the cortisol levels. The blood was collected before anesthesia, and after 1, 2, 4, 8, 12 and 24 h. The authors determined that there is no significant correlation between cortisol levels and preoperative pain (stressed state).

Oswald et al. [68] performed a study to determine the associations between cortisol response and personal traits to chronic stress. The authors inducted 68 healthy individuals who went under controlled laboratory psychological stress tests. The level of plasma cortisol was determined using radioimmunoassay. The authors found that there is a significantly positive correlation ($t = 6.79$; p-value<0.0001) between cortisol levels and induced stress as subjects with higher stress had higher peak cortisol ($M = 18.3$ μg/dL, SD = 10.8 μg/dL) than subjects with lower stress ($M = 12.1$ μg/dL, SD = 5.8 μg/dL).

On the contrary, Herbert et al. [69] studied the plasma cortisol levels at baseline, pre- cold-pressor task (CPT) and post-CPT stimuli in African American (AA) and non-Hispanic White (NHW) adults with knee OA in 91 subjects. An intravenous catheter in the arm was used to measure plasma cortisol levels at three different times; at baseline, before CPT and 20 min after CPT. The cortisol levels were found to be negatively associated with pain (stress) intensity ratings in NHW subjects ($\beta = -0.54$; p-value = 0.001) but had no association in AA subjects.

McQuaid et al. [70] determined the relationship between plasma cortisol and oxytocin with stress, depression, anxiety, and mood of 67 undergraduate females. An additional 18 women were also recruited as a control group. The authors used radioimmunoassay (RIA) to determine the levels of plasma cortisol. Level of stress was determined using the 28-item Stress Appraisal Measure (SAM) scale, depression was measured using 21-item Beck Depression Inventory (BDI), State anxiety was calculated using Spielberger State-Trait Anxiety Inventory (STAI) while the mood was assessed using 41-item Positive and Negative Affect Schedule (PANAS). The analysis showed no significant relationship between stress and stress-related outcomes with plasma cortisol and oxytocin.

Serum cortisol

Ortega et al. [71] examined the cortisol levels in serum before and after pelotherapy in 21 patients with knee OA using commercial enzyme-linked immunosorbent assay (ELISA) kits. The authors determined that OA patients showed significantly elevated serum cortisol levels due to post-treatment perceived pain (stress). Similarly, Tonuk et al. [72] investigated serum cortisol after 10 therapy sessions in 44 knee OA patients. The serum cortisol levels were determined in venous blood samples using the chemiluminescence assay method. The authors concluded that there is a positive correlation ($r = 0.355$, p-value = 0.031) between the change in serum cortisol levels and the change in pain (stress) score.

Bertollo et al. [73] determined the relation of levels of serum cortisol and stress in individuals diagnosed with major depressive disorder (MDD). For this study, the authors recruited 17 MDD patients and 17 healthy individuals as control subjects. Stress levels were determined by adapted 24-questions of the Checklist-90-R manual while serum cortisol was analyzed using the chemiluminescence method. After the analysis, the authors determined that level of cortisol in the body is strongly correlated with stress and depression. They observed that the cortisol levels ($r = 0.70$, p-value <0.05) were significantly high in the stressed individual with MDD than in the control group.

Schaffter et al. [74] studied whether serum cortisol levels measured in patients after acute myocardial infarction (MI) is predictive of PTSD at three and 12 months. The authors included 106 patients with MI and a higher risk of developing post-MI PTSD symptoms. The posttraumatic stress was determined using the German version of the Clinician-Administered PTSD scale (CAPS). Cortisol levels were measured by analyzing the blood samples using an electrochemiluminescence immunoassay on a Cobas analyzer. The Hierarchical regression analysis showed lower cortisol levels (thus, negative correlation) are associated with severe PTSD symptoms at 3- and 12-month post-MI (B=−0.002, p-value =0.042 and B=−0.002, p-value =0.043, respectively).

Other sources of cortisol

Pearlmutter et al. [75] investigated the relationship of sweat cortisol concentration with stress. The author collected sweat samples of 48 college-age athletes and determine the levels of cortisol in the sweat using an ELISA kit. To determine the stress levels, the authors used Kessler 10 Psychological Distress Scale (K10). The authors determined that there was no significant direct relation of cortisol to stress levels of male athletes while a significant positive correlation between the apocrine-dominant sweat cortisol and destress levels of female athletes.

Wu et al. [76] assessed the association of perceived stress levels with the present and subsequent cortisol concentrations in an individual's fingernails. The authors recruited 51 medical students and collected fingernails samples on the 15th (FD15) and 45th (FD45) day of the experiment. The perceived stress was measured using the 10-item Perceived Stress Scale (PSS) while the ELISA kit was used to determine the levels

Table 2
Summary of studies determining correlation between cortisol levels and stress.

Cortisol	Refs	Year	Sample size	Measurement Technique	Correlation
Salivary	[46]	2019	250	Radioimmunoassay	Positive
	[47]	2005	64	Radioimmunoassay	Positive
	[48]	2002	40	Radioimmunoassay with a time-resolved fluorometric detection	Positive
	[49]	2016	31	Cotton Salivettes (Salivette kit)	Positive
	[50]	2020	52	Salivette kit	Not significant
	[51]	2020	12	Salivette Code Blue	Positive
	[52]	2020	112	Salivette kit	negative
	[53]	2020	79	ELISA kit	Positive
	[54]	2020	42	ELISA kit	Not significant
	[55]	2020	53	Salivette kit	Positive
Hair	[56]	2019	57	Salimetrics high-sensitivity cortisol EIA kit	Not significant (less or low stress) / Positive (high stress)
	[57]	2016	270	Liquid chromatography-tandem mass spectrometry (LCTMS)	Positive
	[58]	2015	174	Liquid chromatography-tandem mass spectrometry (LCTMS)	Positive
	[59]	2013	55	Enzyme-linked immunoassay (ELISA) kit	Positive
	[60]	2019	75	ELISA kit	Positive
Urinary	[62]	2003	32	Radioimmunoassay	Negative
	[63]	2016	110	Polypropylene container	Positive
	[64]	2006	28	Polypropylene container	Not significant
	[65]	2014	80	ELISA kit	Not significant
	[66]	2011	104	Radioimmunoassay	Not significant
	[67]	2021	6878	Liquid-Chromatography Mass Spectrometry (LC-MS/MS)	Positive
Plasma	[68]	2001	102	From a cannula inserted in the forearm vein	Not significant
	[69]	2006	68	Radioimmunoassay	Positive
	[70]	2017	91	Intravenous catheter	Negative
	[71]	2016	85	Radioimmunoassay	Not significant
Serum	[72]	2017	21	Commercial enzyme-linked immunosorbent assay (ELISA) kits	Positive
	[73]	2016	44	Chemiluminescence assay	Positive
	[74]	2020	34	Chemiluminescence assay	Positive
	[75]	2021	106	Electrochemiluminescence immunoassay	Negative
Sweat	[76]	2020	48	ELISA kit	Positive
Fingernails	[78]	2018	47	ELISA kit	Not significant
	[79]	2018	51	ELISA kit	Not significant (15 days) /Positive (45 days)

of cortisol. The authors reported no significant association of stress with fingernail's cortisol levels at day 15 while a significant and positive correlation of 45th day's cortisol levels were found with stress. The author concluded that perceived stress was not associated with present cortisol levels, but positively with subsequent cortisol levels. Thus, their findings suggest that cortisol in the fingernails could be indicative of stress exposure in past.

Similar results are also reported by Doan et al. [77]. The authors collected fingernails from 47 African American students (minority population) with low income. The nails were collected at the start of the study and then 2 weeks after the study had begun. Perceived stress was measured using Perceived Stress Scale (PSS), academic stress questionnaire and self-regulation questionnaires. The author found no correlation between perceived stress and fingernails' cortisol levels.

Conclusion

Overall, the most frequently used cortisol for monitoring and detection of physiological as well as psychological stress is salivary cortisol. The literature review also revealed that, in most cases, cortisol levels within the body is either positively or negatively related to stress. This correlation highly depends on the source that is used to measure cortisol levels. Stress is mostly positively related to cortisol levels determined in hair, saliva, and serum. Alternatively, there is also some contrary relationship reported in the previously reported literature. For example, Schmalbach et al. [49] reported a negative correlation of stress with cortisol levels in the saliva. When determining correlation using plasma and urinary cortisol, different studies report contradictory results i.e., 18 reported positive, 4 negative and 10 reported no significant relationship of stress with cortisol levels. Table 2 summarizes the studies reviewed to determine the correlation between cortisol levels and stress in this section.

Application of cortisol detection in clinical research and practice

Cortisol concentration in the body is associated with many clinical outcomes such as, hypertension, dyslipidemia, depression, and anxiety. There are several somatic health factors, chronic and acute stressors, and psychopathological factors that affects the concentration level of cortisol. A detail list of these factors has been reviewed by Wester et al. [79]. In terms of stress monitoring, the different concentration of cortisol can be indicative of cardiometabolic status, chronic stress, and/or psychopathology factor. Each factor is discussed in the detail as follow.

Cardiometabolic status

Cardiometabolic factors (such as obesity, hypertension, diabetes, and dyslipidemia) are highly associated with increased levels of cortisol [80]. Manenschijn et al. [81] and Feller et al. [82] found a positive correlation of cortisol levels with diabetes type 2 disease in elderly population. Increased level of cortisol is also found in patients with myocardial infarction as determined by the Pereg et al. [83]. Stalder et al. [84] showed the relationship between higher cortisol levels and adverse metabolic syndrome. Several studies [85–88] has shown positive correlation of cortisol levels and obesity.

All together, these findings strongly suggest that a long-term cortisol exposure increases the risk of worsen cardiometabolic status and needs to be monitored thoroughly.

Chronic stress

Both psychological and physical stress can result in hyperactivation of HPA axis which results in release of cortisol hormones in the body. The relation of HPA activation and perceived stress is complex as conflicting results are reported in the literature. Kalra et al. [89] reported negative relationship while Karlen et al. [90] reported positive association of cortisol concentration with perceived stress. On the contrary,

Table 3
List of anti-bodies-based cortisol mechanisms along with their minimum detection limit, advantages, and drawbacks.

Method	References	Lowest Detection Limit	Advantages	Drawbacks
Enzyme-Linked Immunosorbent Assay (ELISA)	[100–102]	0.25 pg/ml	Highly sensitive, stable reagents and robust	Requires enzyme labeling
Polyaniline protected gold nanoparticles (PPAuNPs)	[104,112]	1 pM	Oxidation-reduction stability and sensitive	pH value of PBS solution might affect the detection accuracy
Lateral Flow Immunosensor (LFI)	[105,106,113]	3.5 μg/L	Highly sensitive and selective, easy to use/interpret, kit available, no special training requires	Semi-quantitative, Uncertain sample size reduces accuracy and precision
Quartz Crystal Microbalance (QCM)	[107,108]	11 pg/ml	High sensitivity and specificity	Environmental noises effects measurements
Chemiresistor Immunosensor	[109,110,114]	1 pg/ml	Excellent binding label-free selectivity of cortisol	Non-specific adsorption of high molecular weighted hormone from saliva sample
Surface Plasmon Resonance (SPR)	[25,111]	1 μg/L	Highly sensitive, robust, simple, reproducible	Pre-treatment procedure like partial purification is required for accuracy

[91] and [92] reported no significant correlation of cortisol levels with stress.

Thus, further research is required to determine the true relation of perceived stress and cortisol concentration. The effect of factors such as, sex, region, age, and sample size on cortisol concentration should also be investigated.

Psychopathology factors

Anxiety and mood disorder are linked to the cortisol levels in the body. Burke et al. [93] determined that cortisol levels take long time to return to baseline level in people with depression as compared to non-depressed population. Veldhorst et al. [94] conducted a small study with patients with major depression disorder and matched the cortisol levels with control group. Authors found increased cortisol concentrations in depressed patients group compared to control group. The traumatic event and post-traumatic stress disorder (PTSD) is also associated with triggering of HPA axis. In literature, mixed results are found about the association of cortisol levels with PTSD condition. Studies like [95] and [96] showed no significant relationship of cortisol and PTSD while [97] and [98] reported significant changes in cortisol concentration in PTSD and non-PTSD groups.

The literature review shows that the association between cortisol levels and PTSD depends on the type of traumatic event, sample characteristics examined and timespan between trauma and cortisol assessment.

Cortisol assessment in laboratory settings

Many immuno-sensing applications of cortisol using anti-cortisol antibodies have been developed for the diagnosis of stress in laboratory settings. In this section, we have reviewed some state-of-art methods of cortisol measurement for stress detection in laboratory settings. Table 3 summarizes the different methods of anti-bodies-based cortisol mechanisms along with their minimum limit-of-detection, advantages, and limitations. Fig. 5 illustrates the different laboratory-based sensors and their functionality.

Enzyme-linked immunosorbent assay (ELISA)

The ELISA method is the most used analytical immunochemistry assay that is based on a specific bond between the antibody and the antigen. The use of ELISA test for detection and quantification of hormones, antibodies, protein, and peptides, has drastically changed the practice of medical laboratories. The high sensitivity, stable reagent, and robust nature of the test make it a reliable diagnostic tool in laboratory settings. In the biology matrices (like plasma, serum, urine, and saliva) ELISA is an essential tool for the detection of different antigens and antibodies.

Weng et al. [99] designed a microfluidic origami sensor with minimum limit of detection of 6.76 ng/mL with an analysis time of 25 min. Zhang et al. [100] conducted a study to investigate the limits of cortisol detection using ELISA kit. The author determined the minimum possible limit of detection to be 0.25pg/ml with response time of 30 min.

The only drawback of this method is that ELISA is highly dependent on the choice of detecting antigen or antibody and requires accurate labeling of the enzymes [101].

Polyaniline protected gold nanoparticles (PPAuNPs)

The PPAuNPs based cortisol biosensor (an analytical device that measures the chemical reaction by producing proportional signal to the concentration of analyte in the measured reaction [102]) was proposed by Arya et al. [103]. The authors deposited the polyaniline protected gold nanoparticles onto the gold electrode to fabricate an electrochemical cortisol detecting biosensor for the detection of stress in a laboratory setting. They used differential pulse voltammetry and cyclic voltametric technique to determine the concentration of cortisol in a phosphate buffer saline (PBS) solution with pH = 7.0. The accuracy of cortisol detection might get affected by varying pH levels. The lowest cortisol detection limit via this method is 1 pM. The results attained by different studies suggested that the PPAuNP based biosensor remains stable and shows repeated oxidation–reduction peaks during repeated scans. Thus, establishing this method as a feasible method for labeling and mediating freely available cortisol (a stress biomarker) in laboratories.

Lateral flow immunosensor (LFI)

Lateral flow immunoassays are used for qualitative, semi-quantitative and quantitative monitoring. A typical LFA is consists of a surface layer that carries the sample from the sample application pad to the conjugate release pad along with the strip that encounters the detection zone and absorbent pad. The current LFAs are easy to use, highly sensitive and highly selective in detecting antibodies or hormones [104]. Another advantage of the LFA platform is that it comes in form of a kit, thus all the necessary items are in the kit and do not require qualified personnel to conduct the test. The results are also easy to interpret and are visible. In conjunction with being a qualitative or semi-quantitative method, the uncertain sample volume affects the overall accuracy and precision of the test [105]. The minimum level of cortisol detection through LFI is 3.5 μg/L.

Quartz crystal microbalance (QCM)

For stress monitoring and other diagnoses of diseases, the QCM is needed to be deposited on some different sensor electrodes as the molecular weight of cortisol hormone is too small for direct detection on QCM. Ito et al. [106] developed a cortisol detecting system with two sensor chips coupled with QCM. The first sensor was for monitoring antigen-antibody interaction while the second sensor acted as a reference to remove the environmental noise (influences). The sensor system was able to detect the minimum limit of 5 pg/mL and response time of 10 min.

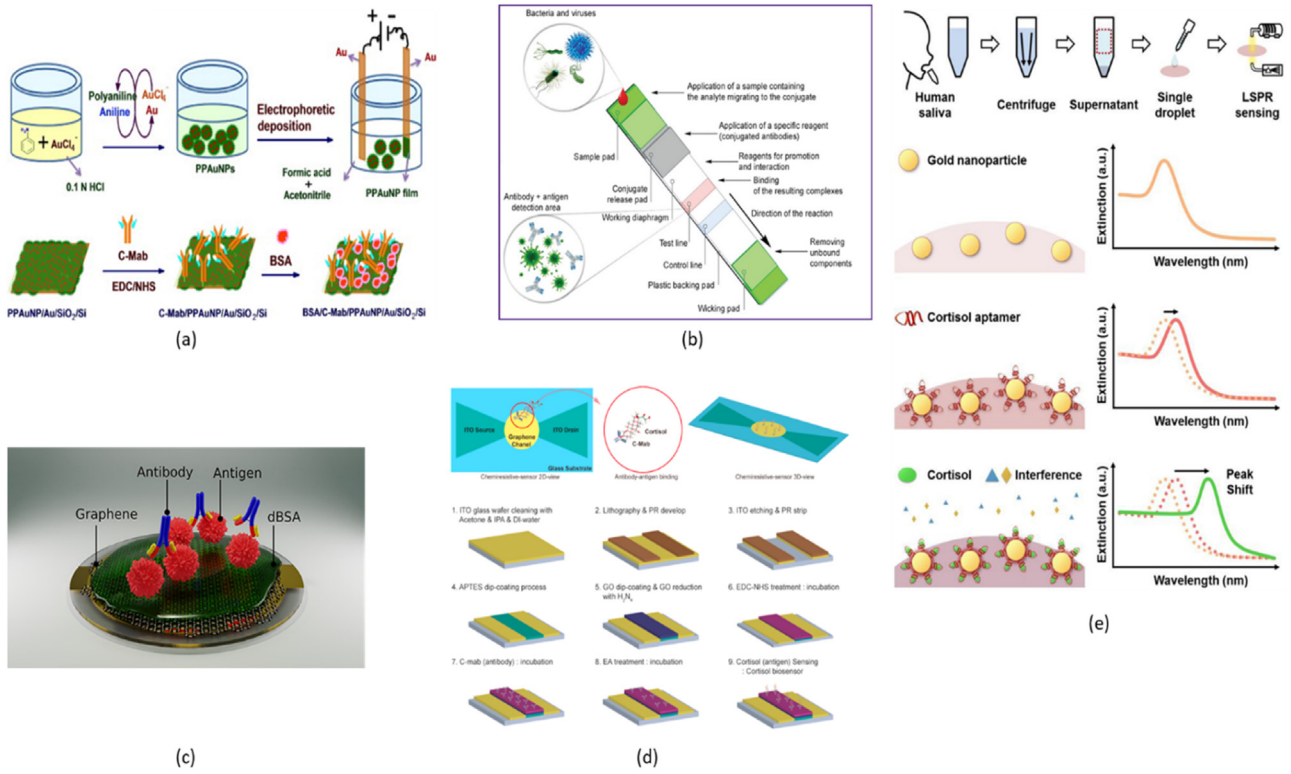

Fig. 5. (a) Shows the fabrication schematics of PPAuNP electrode; reproduced from [104] (b) Represents the schematics of LFI mechanism; reproduced from [106] (c) illustrates the graphical abstract of working of QCM; reproduced from [107] (d) is schematics of chemiresistor sensor fabrication; reproduced from [109] and (e) shows the steps involve in the sampling and detection of salivary cortisol using SPR; reproduced from [111].

Hampitak et al. [107] customized the QCM by coupling it with graphene biointerface sensors (G-QCM) to quantify antibodies in collected serum. Both the studies adopted the QCM method to perform sequential analysis. Cortisol bonded with bovine serum albumin was used as a tracer to monitor the antibody-antigen interaction. The minimum limit of cortisol detection using the QCM method was found to be 11 pg/mL. The sensitivity and specificity results of cortisol detection were comparable with those of ELISA, thus indicating that QCM based sensory system has a great potential for active stress monitoring and disease diagnosis field.

Chemiresistor immunosensor

The chemiresistor immunosensors are frequently used for the detection of stress biomarkers (cortisol) in saliva [108]. Kim et al. [108] demonstrated a cortisol monoclonal antibody (c-Mab) immobilizer on reduced graphene oxide (rGO) channel to work as chemiresistor immunosensor. The authors identified and characterized the cortisol levels based on change of the resistance in the rGO channel. The I-V (current-voltage) characterization of the rGO chemiresistor exhibited the limit of detection of 10 pg/mL.

The recent analytical approaches involve the collection, processing, transportation, storage, analysis, and reporting steps. To facilitate point-of-use salivary cortisol measurement, a carbon nanotube was functionalized with cortisol-3-CMO–NHS ester material and a monoclonal antibody was lighted to develop a chemiresistor immunosensor receptor by Tlili et al. [109]. The immunosensor demonstrated admirable binding selectivity for cortisol hormone (label-free). The minimum detection limit that can be achieved by a chemiresistor immunosensor is 1 pg/mL.

The only disadvantage of this sensing technique is the non-specific adsorption of mucin (high molecular weighted particles) from a saliva sample.

Surface plasmon resonance (SPR)

The surface plasmon resource sensor is constructed using gold nanoparticles. This method of cortisol detection is highly sensitive, robust, simple, and reproducible as antibodies are immobilized using polycarboxylate-hydrogel based coatings for capturing cortisol. The SPR sensor displays excellent cortisol detection capabilities over the wide range of concentrations values. The minimum detection limit using this procedure is 10 μg/L [25].

Jo et al. [110] developed a localized SPR (LSPR) to detect free cortisol with high selectivity in the saliva sample. The LSPR sensor was constructed using different sized gold nanoparticles and was constructed using steps shown in figure 5(e). The authors reported the minimum limit of detection of 0.2 μg/L (0.1 nM in 5μL droplet).

For extraction of cortisol from a bio-fluid (such as blood, urine) that contain a high number of other enzymes or proteins, a pre-treatment procedure like partial purification of these samples is required for accurate detection.

Conclusion

All these systems are portable, highly sensitive, smaller in size than conventional sensors, low cost and provide comparable results with standard electrochemical analyzers. Among the mentioned cortisol measurement methods, the ELISA has the highest sensitivity (1.63 μA M^{-1}), can detection presence of cortisol as low as 0.25 pg/ml, have great reagent stability and is easily reproducible. Along with all these advantages, these systems require multiple steps that should be performed

Table 4
List of Immobilizing Matrix-based cortisol detecting mechanisms along with their properties, advantages, and drawbacks for ambulatory assessment.

Immobilizing Matrix	Refs	Detection technique	Range	Response time	Advantages	limitations
Self-Assembled Monolayer (SAM) membrane and variants	[115]	Electro-chemical immunosensor	0.1 to 10 ng/ml	35 min	Portable, highly sensitive, smaller in size, low cost and accurate	Require multiple steps for accurate detection, expert needed, not user-friendly
	[116]		0.036 to 36 ng/ml	30 min		
	[117,118]		10 pM to 500 nM	30–35 min		
Nitrocellulose membrane and variants	[119]	Lateral flow immunoassay (LFIA)	3.5 ng/ml to 1280 ng/ml	5 min	Portable, handheld devices with highly correlated results	For quantification, bulky reading equipment is required
	[120]		0.9 ng/ml to 25 ng/ml	20 min		
	[121,122]	Colorimetric LFIA	0.3 ng/ml to 60 ng/ml	30 min	Simple, portable, easy to use and best for ambulatory settings	Require 3D printer for printer the adapter and cartridge holder
Platinum Electrode	[126,123]	Electro-chemical immunosensor	0.1 ng/ml to 100 ng/ml	5 min	Portable, handheld devices with highly correlated results	Require the application of 9 V onto the skin for 2 min
Immune-chromatographic chip	[124]	Calorimetric chromatography	1 ng/ml to 10 ng/ml	25 min	Portable, handheld, reusable with a monitor and disposable test strips	Indirect measurement of the cortisol
	[127,128]	Colorimetric LFIA	1 ng/mL to 70 ng/mL	15 min		
Polystyrene pad	[129]	Electro-chemical immunosensor	0.4 ng/ml to 11.3 ng/ml	15 min	Reusable sensor as have a disposable disk	Bulky reading equipment is required
Without any immobilizing antibody or enzyme	[130]	Functionalized nanoparticles	30 pg/ml to 10 μg/ml	2.5 min	Highly sensitive against cortisol hormone and provides strong correlation with ELISA kit	Only proof-of-concept devices, not available to end-users
	[125]	MIP-based electrochemical transistor	0.01 μM to 10 μM	<1 min		

carefully for accurate detection thus making them less reliable, non-user-friendly and not suitable for home base care systems, thus far.

Cortisol assessment in point-of-care/ambulatory settings

In this section, we reviewed the literature investigating ambulatory cortisol measurement methods and devices. Most of the proposed devices are only proof-of-concept and have either ability or potential to assess the stress on the go in a real-life environment. Thus, there is a lot to improve before these methods could be translated to point-of-care or home-based devices. Following are some cortisol assessment technologies categories based on the cortisol immobilizing method used for point-of-care analysis along with their properties, advantages, and limitations. Table 4 summarizes all the literature reviewed under this section. Fig. 6 shows the most popular techniques in the literature for real-time cortisol detection.

Self-assembled monolayer (SAM) membrane and variants

The SAM membrane is a part of an electrochemical based immunosensor that is frequently used to detect the presence of glucose-oxidase-cortisol in saliva samples. These sensors are reusable and highly sensitive as well as detection accuracy highly corresponds with lab-based ELISA. The cortisol detection ranges from 0.1 to 10 ng/ml with a response time of 35 min [114].

Several variants have been proposed to improve the SAM based immobilizing technique. Cruz et al. [115] proposed SAM modified with interdigitated electrodes (IDEs) for the detection of monoclonal anti-cortisol antibodies. The cortisol detection range is slightly improved from the previous device and is between 0.036–36 ng/ml with a response time of 30 min.

Vasudev et al. [116] and Kaushik et al. [117] also proposed an electrochemical immune-sensor using miniaturized potentiostat interfaced with low temperature co-fired ceramic (LTCC) microfluid and modified SAM with IDEs as immobilizing matrix. Both the systems detect the monoclonal anti-cortisol antibodies in saliva and plasma samples. The reported detection range by both the studies is 10 pM to 500 nM with a response time of 30–35 min.

Nitrocellulose membrane and variants

Some lateral flow immunoassay (LFIA) based devices use nitrocellulose membrane as immobilizing matrix for cortisol detection. Such devices capture anti-cortisol-IgG biomolecule for stress monitoring via plasma samples. The detection range of these devices ranges from 3.5 ng/ml to 1280 ng/ml with a response time of 5 min [118]. Shirtcliff et al. [119] designed LFIA based cortisol measuring device that detects salivary cortisol levels with high accuracy. The detection range of the device is 0.9 ng/ml to 25 ng/ml with a response time of 20 min. The device is commercially available for the end-users. Both devices have portable readers, but the overall equipment is a bit bulky.

Choi et al. [120] and Zangheri et al. [121] proposed a colorimetry based LFIA with a smartphone adaptor device that takes the saliva sample on a strip and reads the color of the strip via mobile phone. Both the devices are used to monitor rabbit anti-cortisol antibodies within the detection range of 0.3 ng/ml to 60 ng/ml. The detection results are achieved within 30 min of sample collection. The colorimetry techniques are simple, portable and suitable for ambulatory use.

Platinum electrode

Electrochemical immunosensing works on the principal of calculating the change in the electrical properties of the electrode (a conductive substrate) due to the depositing of any analyte on the surface that is functionalized with antibodies (sensitive to that analyte). The two common method of electrochemical analysis are Electrochemical Impedance Spectroscopy (EIS) and Cyclic Voltammetry (CV) [125].

The electrochemical immunosensors with platinum electrodes as immobilizing matrix are used to detect competitive binding of corticosteroid and corticosteroid-peroxidase conjugate with undisclosed antibodies in saliva samples. These immunosensors are a portable and handheld device that produces highly correlated results with plasma constituents. Measurements can be taken repeatedly after 5 min but require the application of 9 V onto the skin for 2 min. The detection range using this sensor is 0.1 ng/ml to 100 ng/ml [122].

Immune-chromatographic chip

The detecting technique of calorimetric chromatography is used in immune-chromatographic chip sensors for glucose-oxidase-cortisol detection. These sensors measure the salivary cortisol levels as an index of neuroendocrine response. These devices are portable and handheld, consisting of a disposable test strip and a monitor. The detecting range of such sensors is 1 to 10 ng/ml with a response time of 25 min [123].

Panfilova et al. [126] developed an immune-chromatographic test for detection of cortisol using saliva samples. The test was conducted on

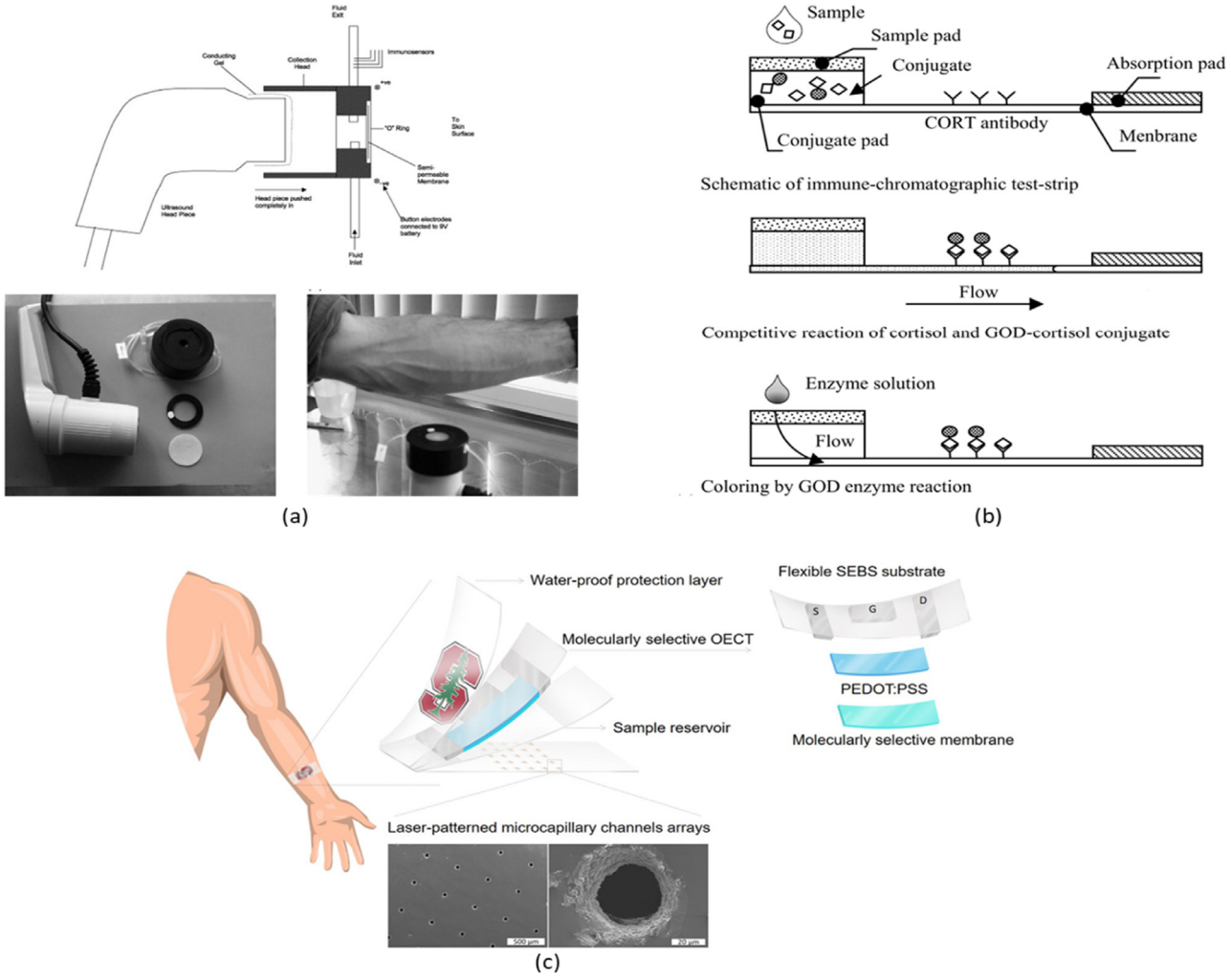

Fig. 6. A platinum based transdermal device with an O ring, membrane, collection chamber and ultrasound delivery device and use of the device in human is shown in (a); reproduced from [123]. (b) demonstrates the working principle of an immune-chromatographic chip; reproduced from [124]. (c) shows the proposed schematics of patch type wearable sweat cortisol sensor; reproduced from [125].

293 saliva samples collected from 10 healthy volunteers. The cortisol detection range reported by the author is 1 ng/mL to 70 ng/mL with the analysis time of 15 min.

Kosicka et al. [127] presented a brief review of different high-performance liquid chromatography techniques for detection of cortisol and cortisone in the urine samples of human. Overall, the only drawback of such devices is it measures the cortisol levels indirectly.

Polystyrene pad (bulky equipment)

Polystyrene pads as mobilizing matrices are used in some electrochemical immune-sensing and optical cortisol detectors. These detectors detect monoclonal anti-cortisol antibodies in saliva within 15 min. Such sensors are developed as automated cortisol immunosensor, incorporating fluid valves and disposable disk chips which makes these sensors reusable. The cortisol detection range using polystyrene pads is 0.4 to 11.3 ng/ml [128].

These sensors allow non-invasive and quantitative analysis with response time compatible with point-of-care applications.

Without any immobilizing antibody or enzyme

As a reaction of cortisol with an immobilizing agent makes the detection process slow, recently few devices are proposed that do not need any antibody or immobilizing enzyme for the detection of cortisol in the given sample. This technique speeds up the overall process of cortisol detection and makes them a potential solution for real-time cortisol detection-based stress monitor. Sanghavi et al. [129] proposed an aptamer functionalized nanoparticle-based cortisol assay device that did not require any antibody or immobilizing enzyme. Their device was able to measure cortisol levels in saliva and plasma within 2.5 min. Their detection range was determined to be between 30 pg/ml to 10 μg/ml. A most recent Molecularly Imprinted Polymer (MIP)-based organic electrochemical transistor patch was proposed by Parlak et al. [124] for cortisol detection in sweat. The patch also did not require any antibodies or immobilizing agents. The patch can detect cortisol levels in the range of 0.01 μM to 10 μM with a response time of less than 1 min.

Devices under this category are highly sensitive against cortisol hormone and provide a strong correlation with results from ELISA kits. The reviewed devices are currently in the development stage and are only proof-of-concept devices but show high potential suitability for ambulatory use.

Conclusion

Among the mentioned cortisol detection techniques, the functionalized nanoparticles and MIP-based electrochemical transistor are the fastest results producing devices that can provide results of cortisol lev-

els within 1 min. These techniques are highly sensitive against cortisol and provides strong corelated results with ELISA kit. The only draw-back is no such device (using these detection methods) is commercially available and is a proof-of-concept. Among the commercially available devices, the platinum electrode-based electrochemical immunosensor has the highest sensitivity (can measure cortisol levels between 0.1 ng/ml to 100 ng/ml) and is easily portable handheld device that provides highly accurate results.

Conclusion and future directions

This review paper highlights the current efforts made in recent years to develop cortisol detection technologies for stress monitoring in real-life. The manuscript presents the feasibility report of cortisol extraction using different bio-fluids, correlation of cortisol levels in different body fluids, the status of laboratory-based reliable cortisol assessment methods and ambulant cortisol measurement techniques along with their analytics. The study showed that the non-invasive methods (like saliva, urine and sweat) of sampling and quantifying the cortisol levels are more feasible than invasive methods (plasma or serum) as invasive interventions induce extra stress and thus affects the true value/state of stress. Furthermore, cortisol secretion is highly correlated to physical and psychological stress, with scarce contrary results. To determine the true relationship between stress and cortisol, further high-quality analysis is required. The effect of sex, ethnicity, and treatments on the association between stress and cortisol levels must also need to be investigated.

The literature search also resulted in several promising strategies, technologies, and devices towards cortisol assessment in daily life. Many studies reported a good sensitivity and specificity of cortisol sampling, showed comparable accuracies in cortisol detection when compared to gold-standard or conventional methods. Some of the devices (such as [121,122] and [124]) were portable, rapid, and easy to use. However, it is be noted that all the devices or technologies discussed in this paper are not commercially available and are only proof-of-concept.

At present, there is no single best analytical method for the development of ambulatory cortisol technology and thus, this field appears to be relatively immature and novel. With significant research efforts and additional investments in the future years, we are hopeful to overcome the remaining procedural and technological hurdles and make a true ambulatory corticosteroid diagnostic wearable stress monitoring device for a home-based care system.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Talha Iqbal: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Visualization. **Adnan Elahi:** Writing – review & editing. **William Wijns:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Atif Shahzad:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision.

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