Patients with severe cutaneous adverse drug reactions have extremely high hair cortisol concentrations that do not correlate with presence of depression.

Eddy M. Zitha

ZTHEDD001

FCDerm (SA), MSc Med (UCT), B.Sc. (Med-Biosciences), MBChB

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Declaration

I, Eddy M. Zitha, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that no part of it has been, is being, or will be submitted for another degree at this or any other university. Prior to registering for the degree, this work had not been reported or published. I authorise the university to reproduce the entire or any portion of the information for the purpose of research in any manner whatsoever.

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ABBREVIATIONS AND ACRONYMS

DissTB	Disseminated tuberculosis
DHEA	Dehydroepiandrosterone
DRESS	Drug rash with eosinophilia and systemic symptoms
EN	Epidermal necrolysis
ELISA	Enzyme-linked immunoassay
GUTB	Genitourinary tuberculosis
HCC	Hair cortisol concentrations,
HIV	Human immunodeficiency virus
MDE	Major depressive episode
M.I.N.I.	The Mini-International Neuropsychiatric Interview
PTB	Pulmonary tuberculosis
SCAR	Severe cutaneous adverse drug reactions
SJS	Stevens-Johnson syndrome,
SJS-TEN overlap	Stevens-Johnson syndrome and toxic epidermal necrolysis overlap,
TB	Tuberculosis,
TBM	Tuberculosis meningitis,
TEN	Toxic epidermal necrolysis.
DHEA/HCC ratio	Dehydroepiandrosterone/ hair cortisol concentrations ratio

ABSTRACT

Background: Hair cortisol concentrations (HCC) +/- DHEA, a depression and stress biomarker has not been studied in severe cutaneous adverse drug reactions (SCAR).

Objective: To determine DHEA/HCC correlation with SCAR-associated depression and compare the ratio with published values.

Methods: Depression was assessed using M.I.N.I. and DHEA/HCC measured in epidermal necrolysis (EN) and DRESS patients at a South African tertiary hospital. PubMed search was conducted for publications documenting DHEA/HCC.

Results: 22/37 participants enrolled were depressed, significantly higher in EN than DRESS. HCC, DHEA or DHEA/HCC were not different between SCAR; depressed versus non-depressed; and presence versus absence of suicidal ideation. DHEA/HCC was unaffected by HIV or TB status. HCC was high in all SCAR patients, regardless of gender. HCC in SCAR was extremely high compared to published healthy controls [309.33 (28.9 - 1835.7) vs. 46.1 (17.7- 153.2), p = <0.01]; depressed subjects [1349.67 (SD 1935.59) vs. 7.26 (SD 0.47), p = <0.01] and depressed HIV positive males [1479.61 (SD 2313.74) vs. 18.02 (SD 9.37), p = 0.0003].

Conclusions: HCC was high and sustained in SCAR irrespective of HIV, TB, or depression status. No association existed between DHEA/HCC ratio and depression. Sustained high cortisol levels potentially impact long-term SCAR-associated outcomes.

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[1]Eddy M. Zitha, MSc, MD; zthedd001@myuct.ac.za; https://orcid.org/0000-0001-6742-8632

[2]Ogheneochuko, U. Oputu, PhD; ogheneochuko.oputu@uct.ac.za; https://orcid.org/0000-0003-2225-2666

[2]Sthomo Mevana, ND, BTECH; qiqa.mevana@uct.ac.za; https://orcid.org/0000-0002-6499-3633

[3]Bukiwe Thwala, MPH; 01480060@wf.uct.ac.za; https://orcid.org/0000-0002-3948-3121

[4,5]Lovemore Mapahla, MSc; lmapahla@gmail.com; https://orcid.org/0000-0002-0146-3299

[3]Aneliswa Mpungose, BCMP; aneliswa.mpungose@uct.ac.za; https://orcid.org/0009-0009-8148-043X

[1]Thuraya Isaacs, MD, MFamMed, MMed; Thuraya.isaacs@uct.ac.za; https://orcid.org/0000-0001-8141-4368

[3]Jonny Peter, MD, PhD; jonny.peter@uct.ac.za; https://orcid.org/0000-0002-2658-0723

[1]Rannakoe J. Lehloenya, B.Sc., MD; rannakoe.lehloenya@uct.ac.za; https://orcid.org/0000-0002-1281-1789

[2]Nonhlanhla P Khumalo, MD, PhD; n.khumalo@uct.ac.za; https://orcid.org/0000-0002-1686-1900

Affiliations:

[1]Division of Dermatology, Department of Medicine, University of Cape Town.

[2]Hair and Skin Research Laboratory, Groote Schuur Hospital, University of Cape Town.

[3]Division of Allergy and Clinical Immunology, Department of Medicine, University of Cape Town.

[4]The Modelling and Simulation Hub, Africa, Department of Statistical Sciences, University of Cape Town, Cape Town, South Africa.

[5]Global Health Department, Division of Epidemiology and Biostatistics, Stellenbosch University, Cape Town, South Africa.

Correspondence: Rannakoe J Lehloenya: rannakoe.lehloenya@uct.ac.za

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Institutional Review Board: This study was approved by the University of Cape Town's Human Research Ethics Committee (HREC REF NO: 790/2020) and carried out in accordance with the Helsinki Declaration.

Keywords: epidermal necrolysis (EN); drug rash with and eosinophilia and systemic symptoms (DRESS); depression; hair cortisol.

ABSTRACT

Background: Hair cortisol concentrations (HCC) +/- DHEA, a depression and stress biomarker has not been studied in severe cutaneous adverse drug reactions (SCAR).

Objective: To determine DHEA/HCC correlation with SCAR-associated depression and compare the ratio with published values.

Methods: Depression was assessed using M.I.N.I. and DHEA/HCC measured in epidermal necrolysis (EN) and DRESS patients at a South African tertiary hospital. PubMed search was conducted for publications documenting DHEA/HCC.

Results: 22/37 participants enrolled were depressed, significantly higher in EN than DRESS. HCC, DHEA or DHEA/HCC were not different between SCAR; depressed versus non-depressed; and presence versus absence of suicidal ideation. DHEA/HCC was unaffected by HIV or TB status. HCC was high in all SCAR patients, regardless of gender. HCC in SCAR was extremely high compared to published healthy controls [309.33 (28.9 - 1835.7) vs. 46.1 (17.7- 153.2), p = <0.01]; depressed subjects [1349.67 (SD 1935.59) vs. 7.26 (SD 0.47), p = <0.01] and depressed HIV positive males [1479.61 (SD 2313.74) vs. 18.02 (SD 9.37), p = 0.0003].

Conclusions: HCC was high and sustained in SCAR irrespective of HIV, TB, or depression status. No association existed between DHEA/HCC ratio and depression. Sustained high cortisol levels potentially impact long-term SCAR-associated outcomes.

Capsule summary

- Hair cortisol correlates with stress and depression. Severe cutaneous drug reaction-associated cortisol and its correlation with drug reaction-associated depression was unknown. We found very high cortisol levels, sustained over months, reflective of reaction severity but not predictive of depression.
- Sustained hypercortisolism may impacts drug reaction-associated long-term systemic and mental outcomes.

Abbreviations and acronyms

dTB (disseminated tuberculosis)

DHEA (dehydroepiandrosterone)

DRESS (drug rash with eosinophilia and systemic symptoms)

ELISA (enzyme-linked immunoassay)

EN (epidermal necrolysis)

GUTB (Genitourinary tuberculosis)

HCC (hair cortisol concentrations)

HIV (human immunodeficiency virus)

M.I.N.I. (The Mini-International Neuropsychiatric Interview)

PTB (Pulmonary tuberculosis)

SCAR (severe cutaneous adverse drug reactions)

SJS (Stevens-Johnson syndrome)

SJS-TEN overlaps (Stevens-Johnson syndrome and toxic epidermal necrolysis overlap)

TB (tuberculosis)

TBM (tuberculosis meningitis)

TEN (Toxic epidermal necrolysis)

INTRODUCTION

Severe cutaneous adverse drug reactions (SCAR), potentially life-threatening reactions that involve the skin, mucosae and sometimes internal organs. The spectrum of SCAR includes epidermal necrolysis (EN) and drug rash with eosinophilia and systemic symptoms (DRESS).(1) Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) form a spectrum of EN, characterised by skin and mucosal pan-epidermal necrosis. In SJS there is ≤10% epidermal detachment, ≥ 30% in TEN, and SJS-TEN overlap lies between these two extremes.(2) EN is linked to significant morbidity and mortality, with in-hospital mortality reaching 29.5% as a secondary diagnosis.(3) EN results in a range of short to long-term morbidities affecting multiple systems, including ophthalmic, genitourinary, respiratory, gastrointestinal, and mental health.(4, 5)

DRESS, by definition involving internal organs presents with a typical rash, fever (>38°C), eosinophilia, atypical lymphocytosis, lymphadenopathy, and internal organ involvement. Dependent on variables like age and comorbidities, mortality can be as high as 10%. DRESS is also associated with a spectrum of short to long-term sequelae including internal organ failure, autoimmunity and mental health disorders.(6, 7, 8)

SCAR is often associated with mental health sequelae. A retrospective Taiwanese population-based cohort study of 212 EN cases and 669 controls found an adjusted hazard ratio of 1.855 (95% CI, 1.587-2.167; $p < 0.001$) for psychiatric disorders.(9) Depression, anxiety and posttraumatic stress disorder were reported in 53%, 43% and 20% of Americans with EN respectively, with 2/3 afraid of taking new medications.(10) Depression was worse in TEN in a study of 24 EN cases, 65% of whom were depressed.(8) A British study found 78% to be depressed and anxious, 46% experiencing nightmares, intrusive thoughts, and flashbacks.(11) Six months post hospitalization, 20% of DRESS were still depressed.(8)

Stress is defined as any stimulus that alters homeostasis for adaptation to the environment, being beneficial in acute stages by aiding coping mechanisms.(12) However, prolonged stress negatively impacts health outcomes, including mental health.(13) Cortisol and dehydroepiandrosterone (DHEA) are produced by the hypothalamic-

pituitary-adrenal axis (HPAA) in a well-coordinated endocrine response to stress. The two hormones collaborate to mediate short- and long-term stress responses to maintain homeostasis. DHEA has anti-inflammatory and anti-glucocorticoid actions, and its serum levels are inversely associated with chronic stress in contrast to cortisol. An increase in DHEA reflects abnormal physiological responses to stress, with shifting of the steroidogenic pathway to DHEA at the expense of cortisol.(14, 15, 16, 17) Their levels in serum, urine, and saliva are used to diagnose endocrine disorders. Due to their opposing effect, cortisol and DHEA has been found to have greater sensitivity in measuring HPAA dysfunction and the net effects of cortisol than each one alone.(18, 19, 20, 21)

Depression is linked to elevated serum cortisol levels due to HPAA hyperactivation, which damages the hippocampus, leading to behavioural changes and depressive symptoms.(22, 23, 24, 25, 26, 27) Chronic stress causes brain degeneration and functional impairment, increasing the risk of depression.(28) Repeated stress can worsen existing mood disorders.(29, 30)

Cortisol incorporation into hair is a promising biomarker for HPAA activity resulting from chronic stress.(31) It's advantages include non-invasiveness, structural and chemical stability on storage, and long-term representation.(31, 32, 33) Hair cortisol concentration (HCC) is independent of hair colour, oral contraceptives, smoking and is proportional to serum concentrations over time. Validated techniques accurately correlate serum and hair cortisol levels, facilitating longitudinal measurement at different time points.(34) HCC has been found to correlate with depression severity in clinically depressed participants.(35, 36) A Chinese study found significant HCC increase following first and recurrent episodes of depression in a female cohort compared to healthy controls, pregnant women with depression also had higher HCC in the first and third trimesters.(37)

The Mini-International Neuropsychiatric Interview (M.I.N.I.) is a validated, structured, clinical interview designed to diagnose psychiatric disorders according to DSM-IV or ICD-10 in epidemiological studies, multicentre clinical trials and non-research clinical settings like ours. It comprises of modules for 17 psychiatric diagnoses. M.I.N.I. requires the interviewee to respond "yes" or "no" to questions.(38)

This study explored if DHEA and HCC correlated with depression in SCAR and compared the hormone's levels to healthy and diseased subjects from published studies. We hypothesised that SCAR trigger a stronger cortisol-driven stress response in hair than has been shown in published studies.

MATERIALS AND METHODS

Study design, participants and ethics

This was a prospective longitudinal study approved by the University of Cape Town's Human Research Ethics Committee (HREC REF NO: 790/2020) as a subset of the existing prospective Immune-mediated Adverse Drug Reactions in African TB HIV endemic settings (IMARI) Registry and Biorepository.(HREC REF NO: 031/2018)(39) For EN and DRESS, IMARI employs RegiSCAR phenotypic validation.(40, 41, 42) All patients gave informed consent for inclusion and publication of the data.

Consenting participants with possible EN and DRESS admitted to the dermatology ward at Groote Schuur Hospital, a tertiary hospital in Cape Town, South Africa between April 2019 and January 2023 were reviewed for the following inclusion criteria:1) age > 12 years, 2) hospitalized due to SCAR necessitating treatment interruption, 3) probable or definite EN or DRESS, 4) ability to navigate the MINI interview, and 5) availability for follow-up at 6 months and 6) consenting for hair sample collection. Treatment for pre-existing psychiatric disease was an exclusion. Demographic and relevant clinical data relating to SCAR phenotype and severity were extracted from the IMARI registry at each time point.

M.I.N.I diagnostic interview

Formally trained M.I.N.I. investigators conducted structured interviews at t_0 (baseline) and t_1 (six months follow-up). Participants who reported suicide risk at any point during the study were referred to a psychiatrist. All questionnaires were administered in the participants' preferred language.

Hair sample collection

Hair samples ranging from 10-50 mg were obtained from participants' scalp using scissors at t_0 and t_1 from the posterior vertex without tugging the hair, then stored at room temperature in aluminium foil until analysis.(43)

HCC and DHEA assays

Hair samples were washed in isopropanol (5 mg hair/mL isopropanol), air dried (seven days), chopped into fine pieces with a scissor and weighed into 2 ml OMNI tubes containing five 2.4 mm metal beads (OMNI International). Hair samples were pulverised in an OMNI Bead Ruptor (USA), and extracted twice with 1mL methanol. Extracts containing pulverised hair were centrifuged at 5000 rpm and each time, 800 µl of the extracts were transferred into a 1.5 ml Eppendorf tubes, and dried at 30 degrees on a miVac Quattro Concentrator (Genevac, England). The dried extracts were reconstituted in 100 µl Salimetrics® assay buffer and analysed within one hour. Samples were analysed using Salimetrics® Cortisol and DHEA Salivary Enzyme Immunoassay Kit (California, USA). All samples were read on a 96 well-plate Variskan Lux Spectrophotometer (Ratastie, Finland).

Quality Assurance (QA) and Quality Control (QC)

Accuracy of data was established by assay of laboratory prepared spikes along with submitted samples. Precision was established for individual sample via duplicate analysis. Coefficient of variation for reported data was less than 20%. All protocol specifications of the supplier of the ELISA kit (Salimetrics) were followed without modification. Sample integrity was preserved and protected by appropriate documentation and storage. Samples which failed QA were resubmitted for repeat assay with corrective actions – dilutions or concentrations. Lab QC samples were run with each batch.

Statistical analysis

The Stata programming language (version 15) was used for all data analysis. To calculate percentages and compare demographics (gender, age, HIV, TB, TB/HIV co-infection, depression and suicide risk) between EN and DRESS, descriptive statistics with mean (standard deviation) were employed to describe the age, which was normally distributed (p-value = 0.68798). We used the Shapiro Wilk test to test if continuous variables were normally distributed; t-test to test age mean difference between phenotype groups; Chi square test of association to test the independence between phenotype groups and other categorical variables; median and interquartile ranges (IQR) to describe HCC (p-value <0.01), DHEA (p-value <0.01) and DHEA/HCC ratio (p-value <0.01), which were not normally distributed; the non-parametric Mann-Whitney test for the difference in measurements between DRESS and EN phenotype groups, depressed and non-depressed groups, and suicidal groups for depressed patients; Chi square test of association to test the association between depression and HIV, TB, and HIV-TB-coinfection. All statistical tests were done at 5% level of significance.

RESULTS

Thirty-seven participants were enrolled, nine with EN and 28 with DRESS. The mean age was 41.56 (SD 9.32) for EN and 42.36 (SD 11.04) years for DRESS; 18/37 (49%) were female. Most participants (81%) were HIV-infected, and 20/37 (54%) were co-infected with HIV and TB. There was no difference in HIV (p=0.49), TB (p=0.26) or HIV/TB (p=0.38) co-infection between EN and DRESS. Depression was diagnosed in 22/37 (59%) participants and was higher in EN than DRESS (89% vs. 50%, p = 0.039), with no difference in suicide risk (p=0.87). **Table 1**. Each participant's raw data is included in **supplementary table 1.**

Table 1. Baseline characteristics of 37 participants with SCAR who were enrolled into the study.

		Phenotype		p-value
Variable	**Level**	**EN (n=9)**	**DRESS (n=28)**	
Gender, n (% age)	Female	2 (22.22)	16 (57.14)	0.068
	Male	7 (77.78)	12 (48.86)	
Age in years, mean (SD)		41.56 (9.32)	42.36 (11.04)	0.8457

HIV	No	1 (11.11)	6 (21.43)	
	Yes	8 (88.89)	22 (78.57)	0.492
TB	No	2(22.22)	12 (42.86)	
	Yes	7 (77.78)	16 (57.14)	0.267
HIV-TB co-infection	No	3 (33.33)	14 (50.00)	
	Yes	6 (66.67)	14 (50.00)	0.383
Depression status	Not depressed	1 (11.11)	14 (50.00)	
	Depressed	8 (88.89)	14 (50.00)	**0.039***
Suicidal risk level	None	7 (77.78)	21 (75.00)	
	Low	2 (22.22)	5 (17.86)	
	Moderate	0 (0.00)	1 (3.57)	0.869
	High	0 (0.00)	1 (3.57)	

n (%) - Total and percentages; **EN**- epidermal necrolysis; **DRESS** - drug rash with eosinophilia and systemic symptoms**. SD-** standard deviation; **HIV**- human immunodeficiency virus; **TB**- tuberculosis; Because age is normally distributed, we utilised the mean (standard deviation) to describe it. The rest of the results were presented as totals with percentages. ***P* < 0.05 EN vs. DRESS.

HCC (p = 0.69), DHEA (p = 0.28) and DHEA/HCC ratio (p = 0.72) were not different between EN and DRESS. Similarly, in depressed and non-depressed participants, there were no differences in HCC (p = 0.40), DHEA (p =1) or DHEA/HCC ratio (p = 0.62). Amongst depressed participants, there were no differences between measured parameters (HCC, DHEA, and DHEA/HCC ratio) or suicide risk. There was also no association between depression and TB (p = 0.32), HIV (p = 0.82) or TB/HIV co-infection (p = 0.94). Table 2.

Table.2. HCC, DHEA concentrations and the DHEA/HCC ratio in SCAR.

2a				
Variable	**Variable group**	**Measurements**		
		HCC in pg/mg, median (IQR)	**DHEA in pg/mg, median (IQR)**	**DHEA/HCC ratio, median (IQR)**
Phenotype, n=37	DRESS (n=28)	422.77 (27.75 -1783.95)	40.2 (23.5 - 93.28)	0.16 (0.03 - 1.32)
	EN (n=9)	135.87 (44.8 - 5012.1)	67.24 (49.3 -210)	1.03 (0.01 - 4.69)
	P-value	**0.6968**	**0.2787**	**0.7233**
Depression status, n=37	Non depressed (n=15)	176.04 (14.3 -1835.7)	65.46 (21.2 -158.1)	0.35 (0.04 -1.19)
	Depressed (n=22)	555.45 (39.58 -1848.7)	45.2 (28.7 - 75.90)	0.11 (0.02 - 1.82)
	P-value	**0.4033**	**1.00**	**0.6206**
Suicidal Risk for the depressed n=22	No (n=13)	695.07 (131.2 -2740.9)	48.4 (21.4 - 67.24)	0.07 (0.01 – 1.03)
	Low (n=7)	44.8 (24.94 -1328.6)	71.4 (29.4 -166.13)	1.82 (0.02 – 4.69)
	Moderate (n=1)	1732.2	29.5	0.02
	High(n=1)	28.9	42	1.45
	P-value	**0.3055**	**0.7958**	**0.4146**
2b				
Variable	**Variable group**	**Depression status**		**P-value**
		Non depressed (n=15)	**Depressed (n=22)**	
HIV	No	4 (26.67)	3 (13.64)	0.320
	Yes	11 (73.33)	19 (86.36)	
TB	No	6 (40.00)	8 (36.36)	0.823
	Yes	9 (60.00)	14 (63.64)	
TB/HIV co-infection	No	7 (46.67)	10 (45.45)	0.942
	Yes	8 (53.33)	12 (54.55)	

n – number of participants; **IQR** - interquartile range; **DHEA** - dehydroepiandrosterone; **HCC** - Hair cortisol concentrations; **EN** - epidermal necrolysis; **DRESS** - drug rash with eosinophilia and systemic symptoms**, HIV** - human immunodeficiency virus; **TB** - tuberculosis; The rest of the results were not normally distributed so we

presented as median and interquartile range. *$P < 0.05$ Depressed vs. non-depressed, Suicidal risk vs. measured parameter in no-suicidal risk and depression vs. HIV, TB, and HIV-TB co-infection and EN vs. DRESS.

We identified seven published studies that assessed HCC and DHEA concentrations in hair, four in healthy controls, three of which reported only HCC and one DHEA. Two in depressed subjects, one in adolescents the other HIV-infected Chinese measured HCC, DHEA and, and their ratio. One study compared HCC to controls in patients with acute myocardial infarction. Each study's finding is summarised in **Supplementary table 2.** The overall HCC in SCAR in this study was significantly much higher compared to the published healthy controls [309.33 (28.9 - 1835.7) vs. 46.1 (17.7- 153.2), p = <0.01]; depressed subjects [1349.67 (SD 1935.59) vs. 7.26 (SD 0.47), p = <0.01] and depressed HIV positive males [1479.61 (SD 2313.74) vs. 18.02 (SD 9.37), p = 0.0003]. This extended to both males and females**. Table 3a.** Findings of each published study is included in **Supplementary table 2.** DHEA concentrations in SCAR were significantly higher for both genders compared to data currently published for depressed adolescents, [80.87 (SD 73 79) vs. 36.5 (SD 1.84), p = 0.01]; healthy females [46.55 (25.3- 77.2) vs. 5.3 (0.5- 11) p = 0.0002]; healthy males [58.5 (21.4- 210) vs. 4.3 (1.2- 6.7), p = 0.0001]; and HIV-infected highly stressed and depressed males 94.20 (SD 85.94) vs 33.71(SD 23.2), p = 0.04]. DHEA/HCC ratio was significantly lower vs. depressed adolescent males [1.29 (SD 1.76) vs. (6.27) p = 0.0001] and depressed adolescent females [2.81 (SD 5.95) vs. 3.84 p = 0.142]. **Table 3c**. However, DHEA was not significantly different when compared to that in depressed adolescents (p = 0.184) and HIV-infected depressed adult males (p = 0.12). **Table 3b.** Similarly, there was no significant difference when comparing DHEA/HCC ratio in SCAR with that in depressed HIV-infected Chinese males (p = 0.126). **Table 3c.**

Table 3. HCC, DHEA concentrations and the DHEA/HCC ratio in SCAR compared to published data

3a: Published Data					**Current study HCC compared to literature (p-value)#**		
Study	Population	Sex (Stress level)	Median (IQR)	Mean (SD)	**Overall** Mean (SD) = 1349.67 (1935.59) Median (IQR) = 309.33 (28.9 – 1835.7)	**Female** Mean (SD) = 1212.7 (1492.24) Median (IQR) = 610.07 (24.94 – 1835.7)	**Male** Mean (SD) = 1479.61 (2313.74) Median (IQR) = 176.04 (39.58 – 1848.7)

Raul et al., 2004. [44]	Healthy adults	All	18 (5.2 -91)		**<0.01***		
Sauvé et al., 2007. [45]	Healthy adults	All	46.1 (17.7-153.2)		**<0.01***		
Karlén et al., 2011. [48]	Healthy adults	F		20.55 (37.66)		**0.0029***	
		M		17.76 (13.34)			**0.0003***
Qiao et al., 2017. [46]	Healthy adults	M (LS)		13.55(5.67)			**0.0005***
		M (HS)		18.02(9.37)			**0.0003***
Kische et al., 2022. [47]	Depressed adolescents	All		7.26 (0.47)	**<0.01***		
		F		7.78 (0.39)		**0.0002***	
		M		6.78 (0.83)			**0.0001***
Faresjö et al., 2020.[48]	Stressed adults with acute MI	F	46.6 (IQR 20.5–100.8)			**0.0123***	
		M	57.8 (IQR 29.0–167.6)				**0.0112***
3b: Published Data					**Current study DHEA compared to literature (p-value)**		
			Median (IQR)	Mean (SD)	**Overall** Mean (SD) = 80.87 (73.79) Median (IQR) = 48.4 (24.3 – 134.3)	**Female** Mean (SD) = 66.80 (57.45) Median (IQR) = 46.55 (25.3 – 77.2)	**Male** Mean (SD) = 94.20 (85.94) Median (IQR) = 58.5 (21.4 – 210)
Kintz et al., 1999. [49]	Healthy adults	F	5.3 (0.5 - 11)			**0.0002***	
		M	4.3 (1.2 - 6.7)				**0.0001***
Qiao et al., 2017. [46]	Depressed HIV+ Chinese	M (LS)		38.97(21.87)			0.1841
		M (HS)		33.71(23.2)			**0.0357***
Kische et al., 2022.[47]	Depressed adolescents	All		36.5 (1.84)	**0.0114***		
		F		29.9 (1.28)		**0.0156***	
		M		42.52(3.29)			0.1152
3c: Published Data					**Current study DHEA/HCC ratio measurement compared to literature. (p-value)**		
			Mean (SD)		**Female** Mean (SD) = 2.81 (5.95) Median (IQR) = 0.11 (0.02 – 1.45)	**Male** Mean (SD) = 1.29 (1.76) Median (IQR) = 0.30 (0.04 – 1.82)	
Qiao et al., 2017. [46]	Depressed HIV+ Chinese	M (LS)	2.87			0.1446	
		M(HS)	1.87			0.1262	
Kische et al., 2022. [47]	Depressed adolescents	F	3.84		**0.042***		
		M	6.27			**0.0001***	

F - female**; M** - male**; IQR** - interquartile range; **HCC** - Hair cortisol concentrations**; HIV** - human immunodeficiency virus**; SD** - standard deviation; **HS** - High stress; **LS** - Lower stress. A single sample to a specified constant value (literature value whether it is mean or median) was presented for the t-test. *$P < 0.05$; **#** Some studies reported their findings as a mean while others reported them as a median and for comparison both are reported for this study.

DISCUSSION

This study aimed to determine whether DHEA and HCC correlate with depression in SCAR, and compare the concentrations in SCAR to healthy controls and diseased subjects from published studies. The major finding of the study was that there was no difference in any of the measurements between the depression status groups. Thus, there is no evidence to support any of the parameters as a marker of depression in SCAR despite 89% and 50% of EN and DRESS participants having significant depression in this study. Depression was found to negatively correlate with HCC and DHEA concentrations in SCAR with no variation when

compared to participants who did not have depression. It is possible that the cortisol-driven stress response in SCAR was so high that it masked any depression-driven differences. Similar to other studies that revealed negative relationship between HCC and depression, this study had some limitations including a limited sample size and a lack of adequate control groups.(50, 51) Independent of depression, confounding factors should also be considered to advance research in this field.

The study found HCC and DHEA to be significantly higher when compared to the existing literature. The overall HCC in SCAR in this study was >10 times higher compared to healthy adults in Raul et al. ($p = <0.01$) and the study of 39 healthy adult students by Sauvé et al. ($p = <0.01$), using similar Salivary ELISA Cortisol kit (Alpco Diagnostics®, Windham, NH) instrument. In contrast, Raul et al. used the Perkin-Elmer Sciex API- 100 Mass Spectrometer. (44, 45) This eliminates the chosen instrument as a confounding factor to explain much higher HCC levels in SCAR. However, the differences between published studies and this study could be attributed to the variations in the populations studies (HIV/TB-infected in SCAR vs. general population or other diseases in the published data); and the time delay (16 to 19 years in the two studies compared to this study) and possible improvements in methodology. It is still possible that the pathogenesis of SCAR itself accounts for this difference. SCAR produces high levels of the proinflammatory cytokine interleukin-6 which has been shown to influence the hypothalamic-pituitary-adrenal (HPA) axis to produce high levels of HCC.(52, 53, 54, 55, 56, 57) This needs further exploration in focused studies.

HCC in depressed HIV-infected participants with SCAR in this study was >80X higher compared to depressed HIV-infected Chinese male adults reported by Qiao et al.(46) Unlike Qiao et al., in this study we used the Salimetrics® Cortisol and DHEA Salivary Enzyme Immunoassay (ELISA) Kit (California, USA) instead of the Liquid Chromatography Tandem Mass Spectrometry (LCMS) (3200 QTRAP, ABI, USA). Regarding demographics, HIV status was a common denominator in these two studies. Differences in age, gender, TB status, and measurement techniques may account for the large disparity. ELISA-based assays are more sensitive than LCMS-based assays for assessing hair cortisol levels.(58)

Limitations

The COVID 19-related lockdown restrictions caused an unavoidable substantially low sample size in this study which restricts the generalizability of the findings. However, the findings provide motivation for larger studies. In addition, a lack of inclusion of healthy study controls resulted in reliance on published data for comparison.

Conclusions

Contrary to published data, there was no detectable correlation between HCC and depression found in EN and DRESS. Instead, SCAR had extremely high HCC compared to published data on healthy and subjects with other diseases.

Recommendations

More research is needed to improve sensitivity and specificity of assays that measure HCC; and to determine whether persistently elevated post SCAR cortisol levels negatively impact medium and long-term outcomes sequelae.

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Supplementary Table 1: Raw data from each participant in the study

BASELINE															SIX MONTH						
ID #	Admission	AGE	Phenotype	Gender	HIV	TB	Depression	Suicide	Hair	Total hair mass(mg) received	Hair Colour	HCC [(pg/mg)	DHEA [] (pg/mg)		Depression	Suicide	Hair	Received Mass(mg)	COLOR	HCC	DHEA
31	24-04-2019	22	DRESS	F	Positive	no	No	No	Long curled	17.3	Black & gold end	1835,7	65,46		No	No	Short straight	8.7	Black & gold end	Min HCC	Min DHEA
39	18-06-2019	68	SJS	M	Positive	PTB	Yes	No	Short afro	6.5	Black	Min HCC	Min DHEA		Lost to follow-up						
50	14-06-2019	32	DRESS	F	Negative	no	No	No	Short relaxed	4.7	Black	Min HCC	Min DHEA		Lost to follow-up						
51	20-06-2019	26	DRESS	F	Positive	dTB	Yes	No	Short afro	10.6	Black	2740,9	24,3		Lost to follow-up (deceased)						
52	20-06-2019	30	TEN	M	Positive	no	Yes	Low	Short curl	16.5	Black	44,8	210		Lost to follow-up						
54	24-06-2019	73	DRESS	F	Negative	No	No	No	Relaxed	4.1	Brown	Min HCC	Min DHEA		Lost to follow-up						
57	12-07-2019	67	DRESS	F	Negative	no	No	No	Straight	10.2	Black & gold end	1178,6	77,2		Lost to follow-up						
63	16-07-2019	63	DRESS	F	Positive	no	No	No	Short curl	4.9	Black	Min HCC	Min DHEA		Lost to follow-up						
71	29-08-2019	26	DRESS	F	Positive	PTB	No	No	Short curl	8.4	Brown	Min HCC	Min DHEA		No	No	No hair sample				
74	03-09-2019	29	DRESS	F	Positive	No	Yes	No	Short curly	4	Black	Min HCC	Min DHEA		Lost to follow-up						
75	04-09-2019	41	DRESS	F	Positive	no	No	No	Short curly	4.4	Black	Min HCC	Min DHEA		Lost to follow-up						
76	06-09-2019	68	DRESS	F	Negative	no	No	No	Straight	3.6	Black/white	Min HCC	Min DHEA		Lost to follow-up						
85	29-10-2019	41	DRESS	F	Positive	PTB	Yes	No	Curly shot	3.5	Black	Min HCC	Min DHEA		Lost to follow-up						
133	30-11-2020	35	DRESS	M	Positive	PTB	No	No	Curly afro	19.4	Brown & black	5760	210		No	No	Long straight	27.2	Black and brown	5760	210
132	22-11-2020	44	DRESS	M	Positive	no	Yes	No	Short curly	130.0	Black	5760	17,9		No	No	Short curly	36.5	Black	5760	210
136	11-12-2020	52	DRESS	F	Negative	no	Yes	No	Long curled	86.1	Black	910,8	48,4		Yes	No	Afro curl	168.9	Black	706,7	210
137	04-01-2021	46	TEN	M	P positive	PTB	Yes	No	Afro curly	34.1	Black	135,87	210		No	No	Short curl	1.2	Black	Min HCC	Min DHEA
139	28-01-2021	54	DRESS	F	Positive	PTB	Yes	moderate	Dread	749.9	Black & gold end	1732.2	29,5		Yes	No	Dread	728	Blond	2435,9	16,0
141	08-02-2021	34	SJS	M	Positive	GUTB	Yes	No	Short curl	23.5	Black	695,07	210		Lost to follow-up						
142	10-02-2021	27	DRESS	M	Positive	TBM	Yes	No	Short curl	55.9	Black	39,58	210		Lost to follow-up						
144	18-02-2021	41	DRESS	F	Negative	PTB	Yes	Low	Short curl	18.4	Brown & black	1328,6	28,7		Lost to follow-up						
164	13-05-2021	33	DRESS	M	Positive	PTB	Yes	No	Long relaxed	222.5	Brown	536,2	38,4		Yes	No	No hair sample				
165	15-05-2021	53	DRESS	F	Positive	dTB	No	No	Long relaxed	149.5	Brown	3338,0	21,2		Lost to follow-up						
167	25-05-2021	41	DRESS	M	Positive	dTB	Yes	Low	Long curled	99.5	Black	574,7	29,4		Lost to follow-up						
183	03-09-2021	39	TEN	M	Positive	PTB	Yes	No	Short curl	88.4	Black	5760	17,4		Yes	No	Short curl	9.9	Black	Min HCC	Min DHEA

184	06-09-2021	53	SJS-TEN	F	Negative	TBM	Yes	Low	Long straight	305.3	Black	24,94	166,13		Deceased						
191	08-10-2021	51	SJS-TEN	M	Positive	GUTB	No	No	Curly afro	57	Black	5760	Min DHEA		Relocated						
194	15-10-2021	28	SJS	F	Positive	No	Yes	No	Curly afro	43	Brown & black	5012,1	49,3		No	No	Curly short	1.2	Black	Min HCC	Min DHEA
196	19-10-2021	56	DRESS	F	Negative	No	No	No	Short afro	172	Black/white	26,6	11,7		No	No	Short afro	5.2	Black	Min HCC	Min DHEA
197	19-10-2021	50	DRESS	F	Positive	PTB	No	No	Afro curly	169	Black	21,6	25,3		Lost to follow-up						
198	20-10-2021	43	DRESS	M	Positive	Lymph node	No	No	Curled	28.4	Black	814,8	134,3		No	No	Short afro	11.6	Black	5760	210
201	26-10-2021	49	DRESS	M	Negative	No	No	No	Straight short	52.2	Black/white	176,04	210		Lost to follow-up						
203	27-10-2021	28	DRESS	F	Negative	Lymph node	No	No	Long relaxed	174.8	Black	14,3	44,7		No	No	Long curl	68.9	Black	5760	210
204	29-10-2021	48	DRESS	F	Positive	no	No	No	Curled	94.2	Black	309,33	109,37		No	No	Short afro	3.4	Black	Min HCC	Min DHEA
222	20-01-2022	45	DRESS	F	Positive	PTB	No	No	Long curled	87.2	Black	9,0	158,1		Lost to follow-up						
226	08-08-2022	59	DRESS	M	Positive	PTB	No	No	Afro dread	334.2	Black	77,4	22,7		Lost to follow-up						
300	31-10-2022	50	TEN	M	Positive	PTB	Yes	No	Short afro	89.2	Black	56,6	58,5		Lost to follow-up						
306	20-11-2022	43	SJS	M	Positive	PTB	Yes	No	Curly afro	46.3	Black	14,18	67,24		Yes	No	Short afro	19.2	Black	13,62	111,78
318	23-11-2022	46	DRESS	F	Positive	no	Yes	No	Long afro	174.5	Black	131,2	19,6		Lost to follow-up						
322	01-12-2022	30	DRESS	F	Positive	PTB	No	No	Short afro	30.2	Black	10,82	210		Lost to follow-up)						
323	05-12-2022	36	DRESS	M	Positive	No	No	No	Short afro	48.6	Black	7,47	6.07		Lost to follow-up						
325	12-12-2022	40	DRESS	M	Positive	No	Yes	Low	Short afro	116.2	Black	19,0	34,5		Yes	No	No hair sample				
326	07-11-2022	47	DRESS	M	Positive	No	Yes	Low	Curly afro	29.6	Brown	32,14	75,90		Lost to follow-up						
331	09-01-2023	48	DRESS	M	Positive	No	Yes	No	Straight	147.8	Black	1848,7	21,4								
333	19-01-2023	28	DRESS	F	Positive	PTB	YES	high	Straight	384.6	Black	28,9	42								
335	23-01-2023	45	DRESS	F	Positive	dTB	Yes	Low	Afro	193.1	Black	3175	71,4		Lost to follow-up						

MIN- (< 10mg of hair sample) hair samples below the minimum sample size for quantification); HCC – hair cortisol concentration; **[ ]** – concentrations; **pg/mg**- picograms (pg) per milligram (mg); **SJS** – Steven Johnson syndrome; **SJS-TEN overlap** – Steven Johnson and Toxic epidermal necrolysis overlap; **TEN** – toxic epidermal necrolysis; **DRESS** – drug rash with eosinophilia and systemic symptoms; **M** – Male; **F** – Female; **HIV** – human immunodeficiency virus; **TB** – tuberculosis. **PTB** – Pulmonary tuberculosis**; dTB** - disseminated tuberculosis; **TBM-** tuberculosis meningitis; **DHEA** - dehydroepiandrosterone; **GUTB-** Genitourinary tuberculosis (GUTB).

Supplementary Table 2: Published studies that report hair HCC, hair DHEA, and the DHEA/HCC ratio and depression.

First author /year	Country	Number of Participants	Gender N (%) mean SD) Male	Female	Age	HCC pg/mg (mean ranges or SD) Male	Female	DHEA pg/mg (mean, ranges/SD) Male	Female	DHEA/HCC ratio (mean/ SD) Male	Female	DHEA/HCC Method or Kit used
Hair cortisol concentration (HCC) in healthy controls												
Sauvé *et al*., 2007. [45]	Toronto	39 healthy students	19 (48.7%)	20 (51.3%)		46.1 (17.7-153.2)		-	-	-		Salivary ELISA Cortisol kit© (Alpco Diagnos- tics®, Windham, NH)
Raul *et al.*, 2004. (44)	France	44 control (Healthy) subjects	17 (38.6%) 0-40 years	27 (61.4%) 40-60 years		18 (5.2 to 91)		-	-	-		Perkin-Elmer Sciex API- 100 Mass Spectrometer
Karlén *et al.*, 2011. [59]	Sweden	99 healthy students	24 (24%)	71 (76%)		17.76 SD13.34. 20.55SD37.66 (Total 19.93 SD33.35)		-		-		RadioImmunoAssay (RIA) (Rabbit Cortisol 3 Polyclonal Antibody, MyBiosource, San Diego, USA)
Hair DHEA concentration in healthy controls												
Kintz *et al.,* 1999. [49]	France	27 control (Healthy) subjects	15 (55%) (17-42 years)	12 (45%)		-		4.3 (1.2-6.7) Total 4.7 (1.2-11)	5.3 (0.5-11)	-		Gas Chromatography- Mass Spectrometry (GC-MS), Hewlett Packard (Palo Alto, CA) GC (6890 series) via a Hewlett Packard (7673) autosampler.
HCC and DHEA concentrations in subjects with depression with HIV infection												
Qiao *et al*., 2017. [46]	Germany	59 Male	All males 33 years (SD8.60)	-		High Stress 19.4 (SD13.15)	Low Stress 14.59(SD9.64)	High Stress 33.71(23.26)	Low Stress 38.97(21.87)	High Stress 1.87.	Low Stress 2.87	Liquid Chromatography Tandem Mass Spectrometry (3200 QTRAP, ABI, USA)
						(p = 0.115)		(p = 0.693)				
HCC and DHEA concentrations in subjects with depressive disorder in adolescents												
Kische *et al*., 2022 .[47]	Germany	985	412 (42%) 18.0 years (SD0.11)	573 (58%); 17.9 (SD0.10		6.78 (SD0.83) Total 7.26 (SD0.47)	7.78 (SD0.39)	2.52 (SD3.29) (SD1.28) Total 36.5 (SD1.84)	29.9	6.27	3.84	Chemiluminescence immuno- assays with high sensitivity (IBL-International, Hamburg, Germany).
HCC in subjects with acute myocardial infarction and controls												
Faresjö *et al*., 2020 .[48]	Sweden	174 Cases	127 (73%); 56.85SD6.2	47 (27%) 56.8SD6.2		57.8 (IQR 29 –168)	46.6 (IQR 20.5–101)	-	-	-		RadioImmunoAssay (RIA) (Rabbit Cortisol 3 Polyclonal Antibody, MyBiosource, San Diego, USA)
		3 055 Controls	1147 (36.3) 56.73SD4.5	2009 (63.7%) 56.73SD4.5		25.9 pg/mg (IQR 17 –52)	0.1 pg/mg (IQR 14–38)	-	-	-		
Mabotha *et al*., 2018 .[60]	South Africa	21 STEMIs	11 (17.2%)	10 (15.6%)		35.18(8.1-209)		-	-	-		Salivary ELISA Cortisol kit© Salimetrics® Cortisol and DHEA Salivary Enzyme Immunoassay Kit (California, USA).
		27 NSTEMIs	10 (15.6%)	17 (26.5%)		17.24(3.7-209)		-	-	-		
		16 Control	0 (0.0%)	16 (25%)		3,32(0.4-11)		-	-	-		

N - total; **SD**- standard deviation; **IQR** - interquartile range; **DHEA** - dehydroepiandrosterone; **HIV**- human immunodeficiency virus; **ELISA**- enzyme-linked immunoassay; **USA**- United States

APPENDICES:

Appendix 1: The Mini International Neuropsychiatric Interview

M.I.N.I.

Mini International Neuropsychiatric Interview

English Version 5.0.0

DSM-IV

Y. Lecrubier, E. Weiller, T. Hergueta, P. Amorim, L.I. Bonora, J.P. Lépine
Hôpital de la Salpétrière - Paris - FRANCE.

D. Sheehan, J. Janavs, R. Baker, K.H. Sheehan, E. Knapp, M. Sheehan
University of South Florida - Tampa - USA.

→ MEANS : **GO** TO THE DIAGNOSTIC BOX(ES) OF THIS MODULE, **CIRCLE NO** IN ALL OF THEM AND **MOVE** TO THE NEXT MODULE

A. MAJOR DEPRESSIVE EPISODE

A1	Have you been consistently depressed or down, most of the day, nearly every day, for the past two weeks ?	NO	YES	1
A2	In the past two weeks, have you been less interested in most things or less able to enjoy the things you used to enjoy most of the time ?	NO	YES	2
	IS **A1** OR **A2** CODED **YES** ? →	NO	YES	
A3	**Over the past two weeks, when you felt depressed and/or uninterested :**			
a	Was your appetite decreased or increased nearly every day or did your weight decrease or increase without trying intentionally ? (i.e., ± 5 % of body weight or ± 3,5 kg or ± 8 lbs., for a 70 kg / 120 lbs. person in a month) IF **YES** TO EITHER, CODE **YES**	NO	YES	3
b	Did you have trouble sleeping nearly every night (difficulty falling asleep, waking up in the middle of the night, early morning wakening, or sleeping excessively) ?	NO	YES	4
c	Did you talk or move more slowly than normal or were you fidgety, restless or having trouble sitting still, almost every day?	NO	YES	5
d	Did you feel tired or without energy, almost every day?	NO	YES	6
e	Did you feel worthless or guilty, almost every day?	NO	YES	7
f	Did you have difficulty concentrating or making decisions, almost every day?	NO	YES	8
g	Did you repeatedly consider hurting yourself, feel suicidal, or wish that you were dead ?	NO	YES	9
A4	ARE **3** OR MORE **A3** ANSWERS CODED **YES** ? (OR **4 A3** ANSWERS IF **A1** OR **A2** ARE CODED **NO**)	**NO**	**YES**	***MAJOR DEPRESSIVE EPISODE CURRENT***
	IF PATIENT MEETS CRITERIA FOR MAJOR DEPRESSIVE EPISODE CURRENT :			
A5a	During your lifetime, did you have other periods of two weeks or more when you felt depressed or uninterested in most things, and had most of the problems we just talked about ? →	NO	YES	10
b	Was there an interval of at least 2 months without depression and/or lost of interest between your current episode and your last episode of depression ?	NO	YES	11
	IS **A5b** CODED **YES** ?	**NO**	**YES**	***MAJOR DEPRESSIVE EPISODE PAST***

→ MEANS : **GO** TO THE DIAGNOSTIC BOX(ES) OF THIS MODULE, **CIRCLE NO** IN ALL OF THEM AND **MOVE** TO THE NEXT MODULE

C. SUICIDALITY

In the past month did you :

C1	Think that you would be better off dead or wish you were dead ?	NO	YES	1
C2	Want to harm yourself ?	NO	YES	2
C3	Think about suicide ?	NO	YES	3
C4	Have a suicide plan ?	NO	YES	4
C5	Attempt suicide ?	NO	YES	5

In your lifetime

C6	Did you ever make a suicide attempt ?	NO	YES	6

IS AT LEAST **1** OF THE ABOVE CODED **YES** ?

IF YES, **SPECIFY** THE LEVEL OF SUICIDE RISK AS FOLLOWS :

C1 or C2 or C6 = YES : LOW
C3 or (C2 +C6) = YES : MODERATE
C4 or C5 or (C3 + C6) = YES : HIGH

NO	**YES**

SUICIDE RISK
CURRENT

LOW	☐
MODERATE	☐
HIGH	☐

Appendix 2: Hair collection SOP Draft Version 02

DIVISION OF DERMATOLOGY	Hair and Skin Research Laboratory

HSR HAIR AND SKIN RESEARCH LAB	**UNVERSITY OF CAPE TOWN** **Division of Dermatology** **Hair and Skin Research Laboratory**	**STANDARD OPERATING PROCEDURE TITLE:** **Hair Sample Collection**

Standard Operating Procedure Number:	HSR001
Effective / Implementation Date:	
Version Number:	**02**
Supersedes Version Number:	

Drafted by:	Mrs A Marthinus	
Author Signature:		**Date: 13 April 2020**
First Reviewer Name:		
First Reviewer Signature:		**Date:**
Final Reviewer Name:	Dr O Oputu	
Final Reviewer Signature:		**Date: 13 April 2020**
Updated by:		
Updaters Signature:		**Date:**
Approved by:		
PI Signature:		**Date of Approval:**

Training Implications

Director	**X**	**Post-Doctoral Fellow**	**X**
Assistant Director	**x**	**Post Graduate Student**	**X**
Research Associate	**x**	**Laboratory technicians and Technologist**	**x**

Review of this SOP would only be required when a formal request for changes is made.

THIS IS A CONTROLLED DOCUMENT. IT MAY NOT BE MODIFIED, COPIED OR DISTRIBUTED WITHOUT AUTHORISATION.

1 PURPOSE:

This SOP describes the hair sample collection procedure.

2 SCOPE:

This SOP is applicable to all those who are authorised to use human hair for study purposes.

3 AUTHORITY AND RESPONSIBILITY:

Laboratory Manager: To ensure that this procedure is implemented and that the staff is trained and fully conversant in this experiment

Laboratory Staff: To follow the procedure as set out in this SOP

Students: To follow the procedure as set out in this SOP

4 MATERIALS REQUIRED

- Aluminium foil
- A pair of scissors
- A pack of envelopes
- A piece of string
- Small specimen bags (10 cm x 15 cm)

5 COLLECTION PROCESS

The sampling does not need to be performed by a physician, but a responsible authority respecting legal, ethical and human rights of the customer. Steps for a hair collection:

5.1. Verify donor identity: When the donor arrives at the collection site, the collector should request photo identification to verify donor identity. If a photo ID is not available, it is acceptable for the donor's supervisor or another employer representative to identify the donor. If the individual's identity cannot be established without a doubt, the collector should not proceed with the collection.

5.2 Hair collection: The sample should be cut from the posterior vertex region of the head, as close as possible to the scalp (see Section 6), since this is the region of least variation in growth rate. If not possible, then the source of the sampling should be described. In general, head hair is estimated to grow at approximately 1.0 cm per month. Sufficient hair must be collected to allow initial testing, followed by confirmatory or re-testing of the sample if necessary. A lock of hair, with the thickness of a pencil is recommended.

5.3 Fill in the required details: The colour, length, body site, and any obvious cosmetic treatment of the hair should be recorded (see Section 7). The donor should be asked to declare all cosmetic treatments in addition to the observed treatments. Root (proximal) and tip (distal) sections of the hair should be clearly defined. If segmental analysis is required, a lock of hair must be fixed before cutting. Head hair is the preferred specimen. Alternative hair (e.g. chest, arm, thigh, pubic, axillary hair) can be collected if head hair is unavailable, if permitted by the company's workplace drug testing policy and the donor.

5.4 Place the lock of hair in aluminium foil: The collector places the lock of hair into a piece of aluminium foil and puts it into a collection envelope.

5.5 Seal the kits: The collector places the tamper-evident label/seal over both collection kit envelopes.

5.6 Instruct the donor to annotate the kit seals: The collector instructs the donor to record her/his initials and date on each of the specimen kit seal.

5.7 Annotate the CCF: The collector completes appropriate sections of the custody control form (CCF) with donor information (e.g. date of birth, telephone numbers), collection information (e.g. date and time of the collection), and chain-of-custody entries, and instructs the donor to sign the CCF.

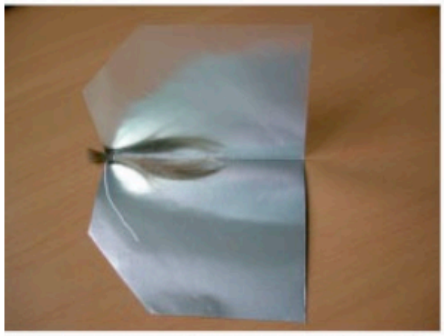
5.8 Check the CCF: The collector checks all copies of the CCF for legibility and completeness. If all copies are legible and complete, the collector then gives the donor a copy of the CCF and permits the donor to leave.

5.9 Prepare specimen for shipment: The collector places the specimen kits along with the laboratory copy (in original) of the CCF in an envelope or box. It is important that the collector ensures each specimen collected is shipped (or picked up by the laboratory's courier) in accordance with the company policy. Samples must be stored and shipped at room temperature, away from direct sunlight or humidity.

6 PICTORAL COLLECTION INSTRUCTIONS FOR HAIR

A lock of hair about the width of a thin pencil, or several locks of hair from the back of the head, are twisted and **fastened with a string**.

The hair is to be cut **just above the skin,** as close to the scalp as possible. The length of remaining segment on the head should be recorded on the request form.



The hair lock(s) are put on separate aluminium foils provided with the root end **exposed at the notched area** of the foil.

Each aluminium foil is to be folded once as shown above.

Insert the aluminium foil into the envelope provided. Seal the envelope.

Fill in the donor's particulars and the required analysis. The donor should sign the declaration. Both hair samples shall be sent to the laboratory in an envelope by post or by the laboratory's courier.

7 CONTROL AND DATA FORMS

Custody control form for hair collection and analysis

Study ID number: .....

Date of birth: .....

Contact details: .....

Date of hair collection ..... Time: .....

Donors Signature: .....

Hair collection details: for laboratory use only

Colour of hair: .....

Length of hair:.....

Body site (head, chest, armpits) : .....

Any obvious cosmetic treatments done in the last year?

.....

.....

Appendix 3: Detection and analysis of cortisol and DHEA from Human hair using an Elisa assay

DIVISION OF DERMATOLOGY	Hair and Skin Research Laboratory

HSR HAIR AND SKIN RESEARCH LAB	**UNVERSITY OF CAPE TOWN** **Division of Dermatology** **Hair and Skin Research Laboratory**	**STANDARD OPERATING PROCEDURE TITLE:** **DETECTION AND ANALYSIS OF CORTISOL FROM HUMAN HAIR USING AN ELISA ASSAY**

Standard Operating Procedure Number:	HSR003
Effective / Implementation Date:	
Version Number:	**01**
Supersedes Version Number:	00

Drafted by:	Sthomo Qiqa Mevana	
Author Signature:		**Date: 15 July 2021**
First Reviewer Name:		
First Reviewer Signature:		**Date:**
Final Reviewer Name:		
Final Reviewer Signature:		**Date:**
Updated by:		
Updaters Signature:		**Date:**
Approved by:		
PI Signature:		**Date of Approval:**

Training Implications

Director	**X**	**Post-Doctoral Fellow**	**X**
Assistant Director	**x**	**Post Graduate Student**	**X**
Research Associate	**x**	**Laboratory technicians and Technologist**	**x**

Review of this SOP would only be required when a formal request for changes is made.

1. PURPOSE:

Analysis of Cortisol in hair samples using the Salimetrics Salivary Cortisol enzyme immunoassay kit.

Cortisol in standards and samples compete with Cortisol conjugated to horseradish peroxidase for the antibody binding sites on a microtiter plate. After incubation, unbound components are washed away. Bound Cortisol Enzyme Conjugate is measured by the reaction of the horseradish peroxidase enzyme to the substrate tetramethylbenzidine (TMB). This reaction produces a blue colour. A yellow colour is formed after stopping the reaction with an acidic solution. The optical density is read on a standard plate reader at 450 nm. The amount of Cortisol Enzyme Conjugate detected is inversely proportional to the amount of Cortisol present in the sample.

2. SCOPE:

This SOP is applicable to all those who are authorised to perform an ELISA assay.

3. AUTHORITY AND RESPONSIBILITY:

Managing Scientist: To ensure that this procedure is implemented and that the staff is trained and fully conversant in this experiment.

Laboratory Staff: To follow the procedure as set out in this SOP

Students: To follow the procedure as set out in this SOP

4. Prerequisites and materials

Please refer to 96-well-plate template filled and approved prior to experiment for the plate lay-out.

4.1. Materials provided by Single Kit:

Microtiter Plate

Cortisol Standards

Cortisol Controls

Cortisol Enzyme Conjugate

Assay Diluent

Wash Buffer 10X

TMB Substrate

Stop Solution

NSB Wells

4.2. Materials Needed but not in Kit:

Precision pipettes (P2-20 uL, 20-200 uL, 100-1000 uL)

Precision multi-channel pipettes (P50-200 uL and P100 – 300 uL).

Vortex

Variskan LUX Plate reader/shaker with maximum 450 nm detection limit

Deionized water

Reagent Reservoirs

Disposable polypropylene tube 30 mL

Pipette tips

Serological pipette to deliver up to 24 mL.

Centrifuge

Heating Block

5. Procedure

5.1. Step 1:

Bring out the Cortisol test kit and allow to stand in room temperature for 1.5 hours.

5.2. Step 2:

Reconstitute samples in 100 uL assay diluent, warm on heating block at 37°C and vortex for 5 minutes.

5.3. Step 3:

Prepare 150 ml of Wash Buffer 1X by adding 135 ml of deionized water to 15 ml of Wash Buffer 10X (for one day use)

5.4. Step 4:

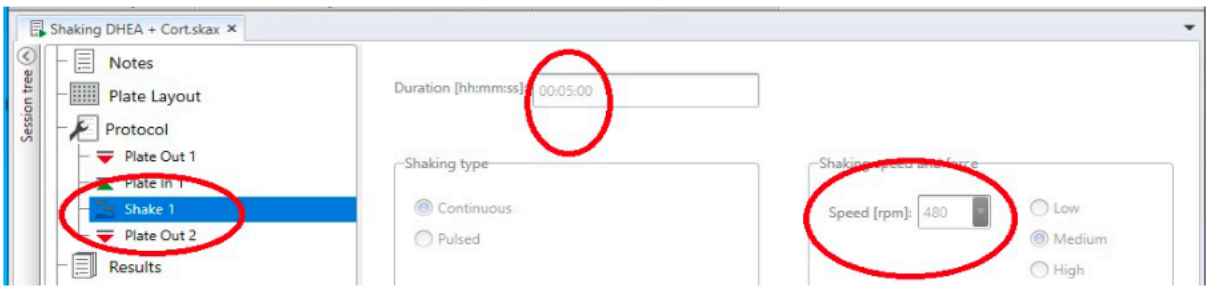
From the microtiter plate, remove strips 1 and 2 from the strip holder and break off the bottom wells (leaving H1 and H2 blank), then break off 2 NSB wells from the strip of the NSB wells to replace in H1 and H2.

5.5. Step 5:

Add 25 uL of standards, controls, and samples into appropriate wells (refer to the 96-well-template), 25 uL of the assay diluent into 2 wells to serve as zero as well as into the 2 NSB wells.

5.6. Step 6:

Prepare Enzyme Conjugate 1:1600 (provided step 1 is complete) by adding 15 uL of the conjugate to 24 mL of Assay Diluent (Scale down proportionally if not using whole plate). Immediately add 200 uL to each well using a multichannel pipette. Seal plate with clear adhesive tape and insert into Varioskan to shake for 5 minutes at 480 rpm by choosing the program “Shaking Protocol for DHEA and Cortisol”. When the Varioskan is done, remove, cover with foil and incubate for 1 hour.

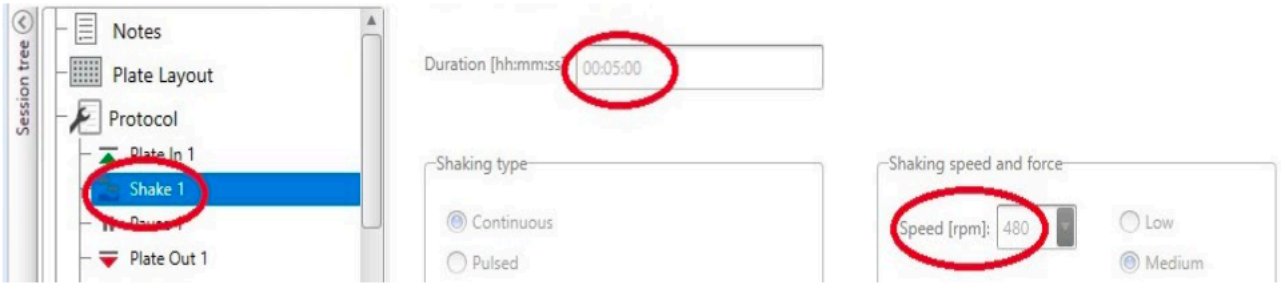


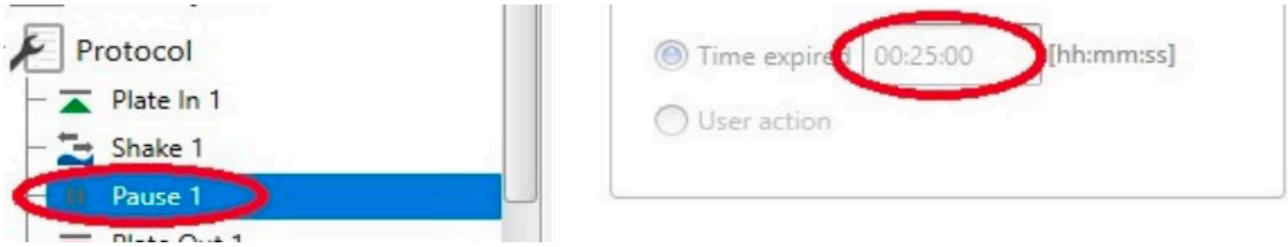
5.7. Step 7:

Wash plate with 300 uL of wash buffer in each well, do this 4 times, blot plate on paper towels each time.

5.8. Step 8:

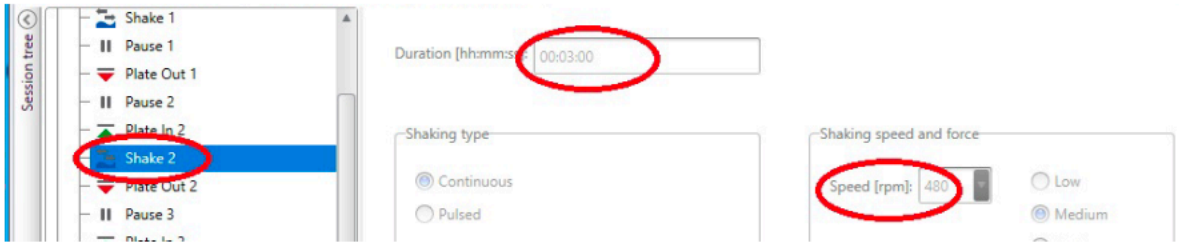
Add 200 uL Tetramethylbenzidine to each well, cover with clear adhesive and insert plate into Varioskan to shake for 5 minutes at 480 rpm using the program "Shaking protocol for Cortisol", this step will be shake 1. This program will include the 25-minute incubation as pause 1.





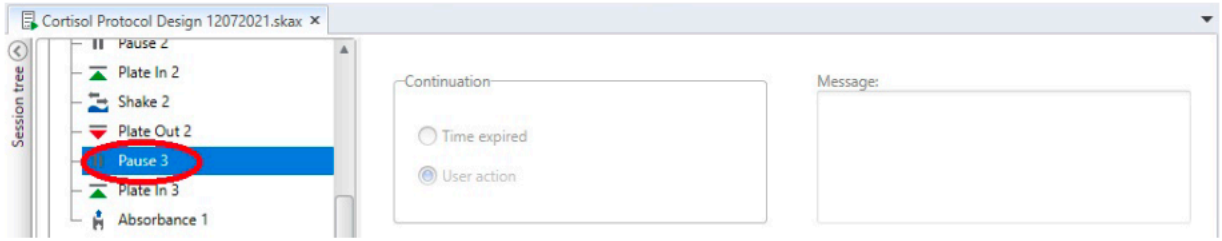
5.9. Step 9:

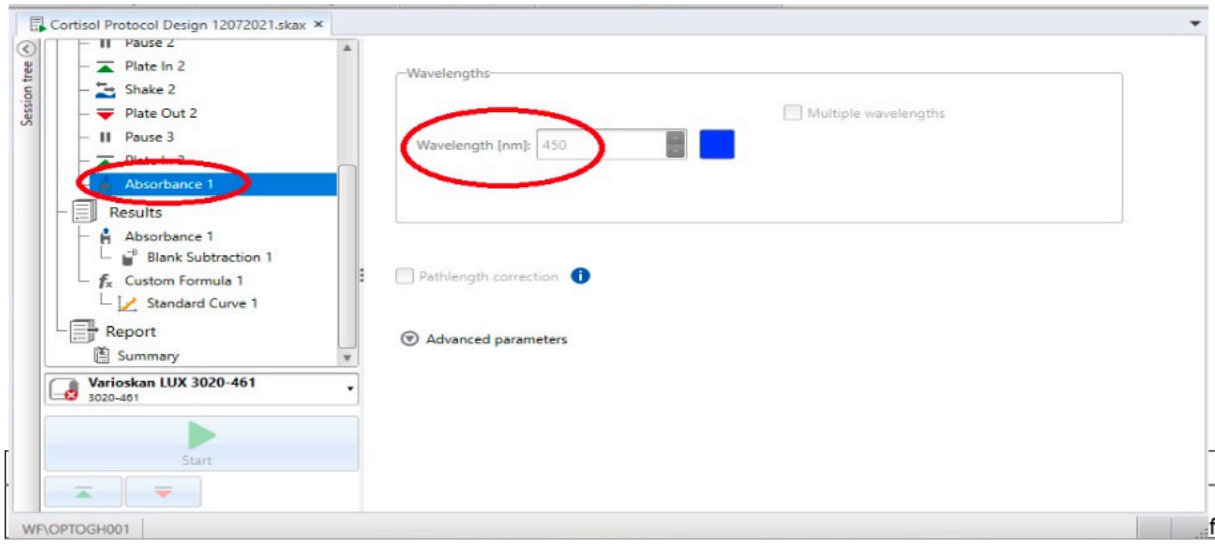
After pause 1 the Varioskan plate will come out for you to add 50 uL of the stop solution to each well, using a multichannel pipette, this will be pause 2. Seal with clear adhesive tape and insert back into Varioskan, press continue for the program to shake for 3 minutes at 480 rpm.



5.10. Step 10:

The program chosen will allow a check at pause 3 to see if further shaking is needed as the solution needs to turn to yellow and to remove the adhesive cover. Press continue for the program to read the absorbance at 450 nm.





6. References

Refer to provided Salimetrics Salivary Cortisol manual.

7. Definitions

TMB: Tetramethylbenzidine

NSB: Non-specific binding

8. Calculations

8.1. Cortisol Enzyme Conjugate

For a 96-well plate, adding 200 uL of the cortisol conjugate gives a final volume of 19 200 uL.

For this, add 15 uL conjugate to 24 mL assay diluent to give a final volume of 24 015 uL.

For 48-wells, adding 200 uL of the conjugate gives a final volume of 9 600 uL.

For this, add 7.5 uL conjugate to 12 mL assay diluent gives a final volume of 12007.5 uL

8.2 Wash Buffer

For a 96 well plate, add 135ml of deionized water to 15ml of wash buffer 10x to make 150ml of 1x wash buffer. This is the minimum dilution that should be made.

Appendix 4: **Consent forms and participant information sheets**

A/Prof Jonathan Peter, Division of Allergy and Clinical Immunology, Department of Medicine, University of Cape Town. Jonny.Peter@uct.ac.za. Tel: 021 650 2210
A/Prof Rannakoe Lehloenya, Division of Dermatology, Department of Medicine, University of Cape Town. Rannakoe.Lehloenya@uct.ac.za Tel: 021 404 3376
A/Prof Sipho Dlamini, Division of Infectious Diseases, Department of Medicine, University of Cape Town, Sipho.Dlamini@uct.ac.za; Tel: 021 406 9111

Participant Information Sheet and Consent Form: IM-ADRs Cases

Introduction

We are approaching you because you have developed an immune mediated adverse drug reaction, such as a rash or blisters on the skin, liver or renal injury, as a result of some of the medication(s) you have been taking. These types of reactions are very common in South Africa. They are very challenging reactions as they end up delaying treatment and making the illness worse. We are collecting information about people like yourself who have been diagnosed with bad reactions severe enough for your doctors to stop or consider stopping your treatment. Please read through this document carefully or listen to whoever is reading this information sheet to you and talk about it to your family if you want. Please ask if there is anything that you don't understand or if there is something that you would like to know.

Why are we collecting your information and samples?

We are collecting information and samples because we want to learn more about why people like yourself take medication and have a bad reaction, while other people who take the same medication tolerate it. We are asking you for permission to collect your clinical information, biological samples and store them for future medical research studies. We plan to use this information in future studies to answer medical and scientific questions relating to reactions like yours. Examples of questions are: How do immune mediated drug reactions occur? What is the best way to diagnose the drug causing the reaction? What increases the chances of developing these reactions? How much time it takes for the drug to be absorbed into the body? How long the drug stays in the body after it has been absorbed? We may use this information and samples to answer other questions not listed above. **We will always request approval from the University of Cape Town, Faculty of Health Sciences, Human Research Ethics Committee before we conduct any studies using the information and/or samples we have collected.**

What information will be collected?

We will collect routine clinical information about your demographics, medical and medication history such as other conditions you may have, drugs you are taking, when they were started, why they were started and if they will be stopped. We will also collect results of your laboratory tests, how your reaction is being treated and duration of your hospitalization. This information will be collected from your paper based and electronic hospital records.

What samples will be collected?

We will collect saliva, blood, a small piece of affected and unaffected skin tissue, urine, stool and hair at different points of the reaction. Time points will include your first presentation at the clinic during the acute stage of the reaction, during the oral drug rechallenge testing at the hospital and then at six monthly intervals during your follow-up clinic appointments. If biological samples of yours have been

taken for diagnostic purposes during previous hospital visits, we may use these in our future studies. We will also like take photographs of your reaction. Photographs will be of various parts of your skin, to document the extent and nature of your skin reaction. Photographs will be stored in our password protected database and access will be restricted to the clinical team. For case adjudication by a small group of experts, photographs will only be connected to de-identified clinical information and not your demographics. We would like to follow-up your case for five years after your reactions through our multidisciplinary clinic.

Procedures and Associated Risks

Blood, Saliva, Urine, Hair and Stool Collection

Wherever possible specimen collection will be performed at the same time as you are being asked to provide a specimen for your routine hospital care. Sample collection will be spaced out and include the following:

- 50 milliliter (mls) of blood (maximum) and 2ml saliva for **genetic testing** will be collected as soon ~~s~~ as possible after you first develop your drug reaction.
- If your doctors decide that it will be safe and beneficial for you to receive oral drug provocation testing, we will collect 50mls blood the day prior to you starting drug rechallenge when all your laboratory results have improved.
- 50ml of blood and a urine sample will be collected if you have a positive reaction to any of the drugs during oral provocation testing, as soon as possible from the onset of your reaction.
- There may be pain, redness or bruising at the site of venipuncture for blood collection.
- If your doctors decide that it will be beneficial for you to receive intravenous corticosteroids (medicine that might help with reducing the symptoms and signs of the positive reaction during the oral provocation test). 50 ml of blood will be collected immediately before the medicine is administrated and after your symptoms start subsiding. Additional 5 X 10mL blood EDTA tubes for ELISpot testing and 1 X 25mL Pax gene tube will also be collected.
- A hair sample (3 to 4 locks of hair)
- A stool samples. Collecting a stool sample will allow us to study your gut microbiome. Disease can change the microbiome and the microbiome can influence disease. We are interested in how the microbiome is affected by drug reactions and also if it may influence the chance of developing a drug reaction. We would like to collect a single stool sample for storage in our biobank, for later characterization of the microbiome.

Skin biopsies and blister fluid (only for severe skin reactions)

If your doctors decide that it is indicated to perform a skin biopsy to aid in the diagnosis of your reaction, we will ask for collection of an additional 3mm size punch biopsy. The biopsies will all be performed by experienced doctors, trained in how to perform these procedures with minimal discomfort. The procedures will be explained to you fully by the treating doctor, and you will be asked to sign the **hospital consent form** for the procedure. The form will list all the risks associated with the procedure. In certain types of reactions there may be blistering or sloughing of the skin. If you are one of these cases, we will ask to collect blister fluid and some of your sloughing skin. Sterile collection of blister fluid will be performed by a trained operator. The rare complications associated with these procedures include reaction to the local anesthetic, bleeding and wound infection.

Liver and Kidney biopsies

Depending on the organ affected by your drug reaction e.g. kidney or liver, your doctors may feel that it is important to take a tissue biopsy of this organ to confirm that it is in fact a drug reaction. If you will a tissue biopsy procedure, we ask that you consent to have one additional tissue sample collected for storage. You will be asked by the treating doctor to sign the **hospital consent form** for the procedure. The form will list all the risks associated with the procedure. You will not undergo liver or kidney biopsy solely for research sample storage purposes, the sample will only be taken when the biopsy is needed for routine care at the hospital.

What are genes?

Genes are the code in the cells of the human body. They are a set of instructions that determine what we look like and control the functioning of our bodies. Our genes control how we break down substances in our bodies, including drugs. Adverse reactions to drugs can sometimes be linked to a specific gene or group of genes.

Why store a specimen for genetic testing?

Currently, it is not known why some people develop immune reactions to drugs, and other people don't. There may be specific genes which increase the risk of developing different types of reactions e.g. liver, kidney or severe skin reactions. Specific genes may increase the risk of immune-mediated drug reactions. We want to store a specimen for genetic testing from you so that we can use this specimen to answer questions about these different immune drug reactions. For example, the specimen may be used to explore the link between genes and blistering skin disease, to work out if only people with specific genes develop reactions, or to answer questions about how genes influence breakdown of medicines in the body. We may use your specimen in studies to answer other questions. We may do focused genetic testing on your sample, where we only study specific genes. We may use your specimen in a Genome Wide Association study. This means that we will look at all of your genes as part of a study. Before doing any genetic research using your specimen, we will obtain approval from the UCT Faculty of Health Sciences Research ethics committee.

If you agree to storage of a specimen for genetic testing, we will ask you some questions about your family ancestry. We will ask you what language is spoken by yourself, your parents and your grandparents, and we will ask you to describe your ethnic ancestry. The reason that we are collecting this information is so that we will be able to identify if particular groups of people are more likely to have particular genes in future studies. This will not be of any direct benefit to you. This information will be used in future studies using your specimens. It may allow us to identify groups of people who need closer monitoring because they are very likely to carry genes that put them at increased risk of drug adverse effects. It may allow us to identify groups of people that require additional screening tests because they are at increased risk of an adverse effect. You can decline to answer these questions if you are not comfortable with sharing this information. This will not affect your clinical care in any way, and you can still choose to contribute specimens for storage. We will not give results of genetic tests to you or to your family.

What are the risks associated with genetic testing?

Genetic material contains information about many different traits. The traits being tested may be heritable which means they may be passed on from generation to generations in the family and might extend to identifiable population or groups. The knowledge of the presence of genetic traits that may increase risk of disease or drug response within a certain population could result in potential loss of confidentiality, discrimination or stigmatization. This means genetic testing can have consequences for you, your family members as well people in your community or demographic. These risks can change depending on the type of questions we choose to answer in our future research studies. If you have any concerns or require more details about the risks, you can discuss it with your doctor or genetics professional. Your saliva samples to be used for DNA analysis will be labelled with a laboratory code to protect your identity and under no circumstances will any information linking you to specific test results willingly be disclosed to any individual or organization.

Do I have to take part?

It is your choice whether you would like your information and specimens to be collected and stored or not. If you do not want to, you do not have to give a reason and you can change your mind at any time. If you decide that you don't want your information and samples to be stored now or later, you will continue to receive the same care.

Where will my information and samples be stored?

Your samples will be stored at the laboratory for allergy and clinical immunology at the University of Cape Town. Some of the samples may be send to laboratories outside South Africa for analysis. Your

identity will be removed from all samples, stored samples will be labelled with a database number. All information collected will be stored securely in a UCT-based database with password protection. Research study results generated from your samples will be published in scientific journals. These will not include any information which identifies you personally.

Will I be paid to take part?

You will not be paid for participation in the registry or for specimen collection. However, once you are discharged from the hospital, we will cover the cost of your transport each time you return to the clinic for six monthlies follow-ups. You will be paid R100 for each visit.

What do I do if I have any further questions?

You are encouraged to ask the investigators should you have any questions or concerns, you can call Professor Rannakoe Lehloenya on 021 404 3376 and/or Professor Jonny Peter on 021 650 2210.

University of cape town human research ethics committee

If you have any queries related to the ethical conduct and approval of this study or would like to report any concerns about study conduct, you can directly contact the Chairperson of the Human Research Ethics Committee at the University of Cape Town Professor Blockman on 021 406 6492.

CONSENT FORM

I.....give the investigator permission to:

(Tick what the patient agreed to)

	Photographs
	Collect 2ml saliva
	Collect 50ml blood and urine at each of the time-points
	Collect stool sample
	Collect hair sample
	Collect skin biopsy (for severe skin reactions)
	Take additional piece of tissue for storage should I require liver or kidney biopsy for routine care
	Collect and store my clinical information for any future research
	Store all my samples for any future research
	Access to existing biological samples

I am aware of all the procedures and associated risks. I understand that I may withdraw at any time without giving a reason and I will continue to receive the same care.

The information sheet and this form have been explained to me. The investigator has answered all my questions. I fully understand what will happen with my information and samples and I consent.

Participant Signature:..... Date:.....

Witness Signature:..... Date:.....

Statement Of Investigators Responsibility

I have explained the nature, purpose, procedures, benefits, risks and alternatives to this research study. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

Researcher's Signature:…….. Date:……..

Appendix 5: Ethics approval letter from the Faculty Research Ethics Committee

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room G50- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

08 December 2020

HREC REF: 790/2020

A/Prof R Lehloenya
Division of Dermatology
G-23 NGSH
Email: - rannakoe.lehloenya@uct.ac.za
Student: eddyzitha@gmail.com

Dear A/Prof Lehloenya

PROJECT TITLE: EPIDERMAL NECROLYSIS AND HAIR CORTISOL: A NOVEL BIOMARKER OF STRESS AND PREDICTOR OF DEPRESSION-MMED CANDIDATE DR EDDY ZITHA SUB-STUDY LINKED TO R031/2018; 270/2020;619/2020

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020 & 06 July 2020.

Approval is granted for one year until the 30 December 2021.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: - Dr Eddy Zitha will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.

Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Appendix 6: Instructions to Authors the Journal of the American Academy of Dermatology (JAAD).

Original Article

A description of the type of study that was done (case series, case-control, cohort, cross-sectional, randomized controlled trial, ecologic, etc.)

Maximum Word Count	2500
Maximum Reference Count	No limit
Maximum Figures/Tables	5
Requires Abstract	200
Capsule Summary two bullet point	50
Required Documents	1. Cover Letter 2. Title Page (including patient consent statement, conflict of interest statement, and funding statement, IRB) 3. Manuscript (Microsoft Word documents only) 4. Separate file figures if applicable (.tif, .eps., or .jpeg) (NOT for tables) 5. CONSORT manuscript checklist (only if applicable)

Appendix 7: Journal of the American Academy of Dermatology (JAAD) manuscript draft.

Journal of the American Academy of Dermatology

Patients with severe cutaneous adverse drug reactions have extremely high hair cortisol concentrations that do not correlate with presence of depression.

--Manuscript Draft--

Manuscript Number:	JAAD-D-23-03156
Article Type:	Original Article
Keywords:	epidermal necrolysis (EN); drug rash with and eosinophilia and systemic symptoms (DRESS); depression; hair cortisol
Corresponding Author:	Rannakoe J Lehloenya, MD Cape Town, Western Cape SOUTH AFRICA
First Author:	Eddy M Zitha, MSc, MD
Order of Authors:	Eddy M Zitha, MSc, MD
	Ogheneochuko U Oputu, PhD
	Sthomo Mevana, ND, BTECH
	Bukiwe Thwala, MPH
	Lovemore Mapahla, MSc
	Aneliswa Mpungose, BCMP
	Thuraya Isaacs, MD, MFamMed, MMed
	Jonny Peter, MD, PhD
	Rannakoe J Lehloenya, MD, PhD
	Nonhlanhla P Khumalo, MD, PhD
Manuscript Region of Origin:	SOUTH AFRICA
Abstract:	Background Hair cortisol concentrations (HCC) +/- DHEA, a depression and stress biomarker has not been studied in severe cutaneous adverse drug reactions (SCAR). Objective To determine DHEA/HCC correlation with SCAR-associated depression and compare the ratio with published values. Methods Depression was assessed using M.I.N.I. and DHEA/HCC measured in epidermal necrolysis (EN) and DRESS patients at a South African tertiary hospital. PubMed search was conducted for publications documenting DHEA/HCC. Results 22/37 participants enrolled were depressed, significantly higher in EN than DRESS. HCC, DHEA or DHEA/HCC were not different between SCAR; depressed versus non-depressed; and presence versus absence of suicidal ideation. DHEA/HCC was unaffected by HIV or TB status. HCC was high in all SCAR patients, regardless of gender. HCC in SCAR was extremely high compared to published healthy controls [309.33 (28.9 - 1835.7) vs. 46.1 (17.7 - 153.2), p = <0.01]; depressed subjects [1349.67 (SD 1935.59) vs. 7.26 (SD 0.47), p = <0.01] and depressed HIV positive males [1479.61 (SD 2313.74) vs. 18.02 (SD 9.37), p = 0.0003]. Limitations

	Small sample size and variability in test methods used. Conclusions Hair cortisol was high in SCAR irrespective of HIV, TB, or depression status. No association existed between DHEA/HCC ratio and depression.