Hair Growth Pathway, A Novel Innovative Method for Assessment and Detection of Chronic Stress in Adult Female Wistar Rats.

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ABSTRACT

Recent research findings have observed the possibility of extracting hair strands to determine long term chronic stress prognosis and its been recognized as an alternative, reliable sample route for chronic stress assessment. The aim of this study is to assess and detect chronic stress exposure in hair using Adult female Wistar rat. A total of 30 female albino rats weighing 100 -170g was used. These experimental animals were divided into four(4) Groups. Group one (1) was control group n=10, Group two (2), =10 was experimental group exposed to physical stressor and Group three (3) n=10 was experimental group exposed to chemical stressor while Group(4) was recovery group from Group three and four(5 each) after the stressor induction. Stress biomarkers analyzed were cortisol, Ghrelin, DHEA, Prolactin, INFG,IL-1B, IL-6, C-reactive Protein, Sodium, Potassium and calcium. Data was analyzed using Independent Sample T-Test, One Way Anova followed by Post Hoc. Values were considered significant at P=0.05. In this study, hair growth pathway detected chronic stress when compared with the baseline during physical stress with sodium (262.45mmol: l:301.19mmol/L). Also, chronic stress was assessed during chemical stress using hair route with calcium, C-reactive Protein, Prolactin, Ghrelin, DHEA. In addition, chronic stress was observed during chemical stress recovery phase when compared with baseline using hair route in calcium, C-reactive protein, Prolactin, Cortisol, DHEA but during Physical Stress Recovery Phase, chronic stress was observed with C-reactive Protein , Prolactin, cortisol, DHEA. In conclusion, this work has revealed that chronic stress could be assessed and detected with Hair Growth Pathway using these stress biomarkers-Sodium, Calcium, C-reactive Protein, Prolactin, Ghrelin, DHEA and cortisol. Therefore, hair Growth Pathway could provide a novel innovative route and could be used in assessment and detection of chronic stress.

Keyword-Hair Growth Pathway, Chronic Stress, Physical Stress, Chemical Stress, Stress Biomarkers.

INTRODUCTION

Chronic Stress exposure is associated with Psychological and biological changes with risk for disease. (Schaafsma *et al.,* 2021). Studies done over the years has observed biological fluids i.e. serum, saliva and urine during laboratory analysis to have acceptable sensitivity during stress assessment (Lightman *et al.,* 2008).This sample method procedure undergoes various logistical challenge i.e. participant compliance, expensive route and participant response to stress may be increased at particular time (Kudiella *et al.,* 2003).However, hair can provide biological information of long term exposure to stress due to its growth rate is about 1cm/month. This can provide a baseline biomarker assessment for period of before and after chronic stress evaluation. Hair has a larger detection period(ranging from days to years),It is non invasive ,easy to be performed and has provided a wider research area on individual stress profiles and promoting more personalized healthcare driving advancements in the field. The aim of this study is to evaluate chronic stress exposure in hair using different stress biomarkers on adult female wistar rat. Stress biomarkers that were used includes; Cortisol, Prolactin, Acylghrelin, C-reactive Protein, Dehydroepiadsterone, Proinflammatory cytokines-Interleukin 1b, Interleukin 6, Interleuken Y, Biochemical parameters- Na^+, K^+ and Ca2+.This research study was carried out at Department of Human Physiology Laboratory, Nnamdi Azikiwe University, Nnewi Campus, Nigeria. A total of 30 female albino rats weighing 100-170g was used. These experimental models was divided into Four (4) groups. Group one (1) was control group, Group two (2) and three (3) was the Experimental Group while Group 4 was Recovery Group. Each of these grouped animals was identified with different color of dyes.

Study Group 1 (Control Group)

This group of animals was handled without stress exposure (control; n=10)

Study Group 2 (Experimental Group 1)

This Group was exposed to a physical stressor for three (3) hours, during morning period for 4 weeks (n=10).

Study Group 3 (Experimental Group 11)

This Group was exposed to a chemical stressor for four (4) weeks (n =10)

Study Group 4 (Recovery stage)

Five (5) of the experimental models from each of the Experimental Group B and C was kept for recovery, for a period of 2 weeks before they were sacrificed.

Laboratory Procedure

Method of Physical Stress Induced.

The method of stress that was used is restraint method of stress. Restraint stress was performed by placing each animal in one – foot ventilated cylindrical tube for a period of 3 hours. This method of stress is probably reported as a wide spread of stress induction. It is also perceived as a severe stressor and induces entire spectrum of known allostic responses (Wei Zhang *et al.,* 2014). PVC cylindrical pipe was cut into 3cm twenty pieces, few dot holes was marked in each of the pipes for ventilation and a rubber black seal was used to close the both side openings. Each of experimental animal was placed inside the pipes for 3hours for a period of 4 weeks.

FIGURE 2: Physical Restraint Chambers Used.

Lead –Induced Stress Method

This experimental Group was ingested with 1/20 of the oral LD50 respectively (LD%) of led acetate in rats =550mg/kg per body weight) This was done alternatively every 2 days for period of Four (4) weeks (Nabil *et al.,* 2012).

Assessment of Stress Using Hair Growth Pathway

To monitor hair growth patterns, firstly, before the stress was induced. A portion of experimental model hair was shaved, collected, washed and then put in a sample bottle, the shaved area was circled with black dye (Craven *et al.,*2001). Subsequently, hair growth was monitored daily by visual examination (during laboratory stressor procedure) for the presence of new unstained hairs. Constant monitory of hair growth weekly was noted and then marked area at the area. After the laboratory stress induced procedure, Regrown hair at the shaved area was shaved, collected, washed and then put in a sample bottle for stress assessment. This hair growth was monitored for 3 weeks during period of chronic stress exposure.

Hair Sample Collection

Experimental model hair was shaved before and after the experimental procedure and was wrapped in a white nylon, labelled with ID study Number and kept in a cool dry cupboard. Hair was washed with distilled water and dried. Then 10mg of washed and dried hair was dissolved in 1ml of 1m Na0H at 70-90 o^c for 30 minutes (Sporkert and Pragst,2000).

All experimental procedures were reviewed and approved by Nnamdi Azikiwe University, Animal Research Ethics Committee Awka. All statistical analyses were carried out using the Statistical Packages for Social Sciences (SPSS Inc. Chicago Illinois) software version 25.0. Comparison of continuous variable was presented with independent Student's and Paired t-test followed by Post Hoc. Statistical tests with probability values less than or equal to 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Table 4.1: Physical Stress Induction on Stress Biomarkers using Hair Growth Pathway

Parameter	**Control (Baseline) Mean ± SEM**	**Physical StressMean ± SEM**	**Mean Difference**	**P alue**
Calcium (mmol/L)	1.75 ± 0.02	2.11 ± 0.20	0.36	0.150
Potassium (mmol/L)	2.19 ± 0.25	2.12 ± 0.16	−0.07	0.830
Sodium (mmol/L)	301.19 ± 7.34	262.45 ± 6.06	−38.74	**0.004***
IFNG (pg/mL)	45.73 ± 11.30	47.22 ± 15.68	1.49	0.940
IL-1β (pg/mL)	196.61 ± 100.48	275.01 ± 56.88	78.40	0.544
IL-6 (pg/mL)	149.15 ± 124.01	166.72 ± 67.58	17.57	0.693
C-Reactive Protein (mg/dL)	1.18 ± 0.17	0.89 ± 0.06	−0.29	0.502
Prolactin (ng/mL)	0.06 ± 0.01	0.72 ± 0.04	0.66	0.607
Cortisol (ng/mL)	4.06 ± 0.25	4.57 ± 2.76	0.51	0.497
Ghrelin (ng/mL)	1.08 ± 0.17	1.18 ± 0.24	0.10	0.744
DHEA (ng/mL)	1.85 ± 0.51	2.12 ± 0.55	0.27	0.732

Table 4.2: Chemical stress Induction on Stress Biomarkers using Hair Growth Pathway

Parameter	**Control (Baseline) Mean ± SEM**	**Chemical StressMean ± SEM**	**Mean Difference**	**P Value**
Calcium (mmol/L)	1.75 ± 0.02	1.65 ± 0.03	−0.10	**0.017***
Potassium (mmol/L)	2.19 ± 0.25	2.68 ± 0.16	0.49	0.140
Sodium (mmol/L)	301.19 ± 7.34	320.01 ± 3.20	18.82	0.061
IFNG (pg/mL)	45.73 ± 11.30	82.25 ± 21.00	36.52	0.175
IL-1β (pg/mL)	196.61 ± 100.48	105.06 ± 172.86	−91.55	0.164
IL-6 (pg/mL)	149.15 ± 124.01	146.15 ± 104.32	−3.00	0.913

C-Reactive Protein (mg/dL)	1.18 ± 0.17	0.11 ± 0.04	−1.07	**0.021** *
Prolactin (ng/mL)	0.06 ± 0.01	0.12 ± 0.07	1.06	**0.003** *
Cortisol (ng/mL)	4.06 ± 0.25	7.40 ± 0.46	3.34	0.253
Ghrelin (ng/mL)	1.08 ± 0.17	0.60 ± 0.10	−0.48	**0.048** *
DHEA (ng/mL)	1.85 ± 0.51	5.09 ± 3.69	3.24	**0.021** *

Data was analyzed using Independent Sample T Test with 95% confidence interval of the Difference P value≤0.05 is considered significant

Table 4.3: Chemical Stress Recovery Phase on Stress Biomarkers using Hair Growth Pathway

Parameter	**Control (Baseline)Mean ± SEM**	**Chemical Stress RecoveryMean ± SEM**	**Mean Difference**	**P Value**
Calcium (mmol/L)	1.75 ± 0.02	1.66 ± 0.01	−0.09	**0.005***
Potassium (mmol/L)	2.19 ± 0.25	2.77 ± 0.19	0.58	0.133
Sodium (mmol/L)	301.19 ± 7.34	310.39 ± 6.63	9.20	0.380
IFNG (pg/mL)	45.73 ± 11.30	36.51 ± 5.38	−9.22	0.491
IL-1β (pg/mL)	196.61 ± 100.48	197.64 ± 107.21	1.03	0.988
IL-6 (pg/mL)	149.15 ± 124.01	154.61 ± 97.24	5.46	0.796
C-Reactive Protein (mg/dL)	1.18 ± 0.17	0.05 ± 0.03	−0.06	**0.019***
Prolactin (ng/mL)	0.06 ± 0.01	1.18 ± 0.07	1.12	**0.001***
Cortisol (ng/mL)	4.06 ± 0.25	9.41 ± 5.62	5.35	**0.016***
Ghrelin (ng/mL)	1.08 ± 0.17	1.24 ± 0.17	0.16	0.505
DHEA (ng/mL)	1.85 ± 0.51	5.73 ± 0.95	3.88	**0.011***

Data was analyzed using Independent Sample T Test with 95% confidence interval of the Difference

P value ≤ 0.05 is considered significant * shows significant

Table 4.4 Physical Stress Recovery Phase on Stress Biomarkers Using Hair Growth Pathway

Parameter	**Control (Baseline)Mean ± SEM**	**Physical Stress RecoveryMean ± SEM**	**Mean Difference**	**P Value**
Calcium (mmol/L)	1.75 ± 0.02	1.75 ± 0.06	0.00	0.906
Potassium (mmol/L)	2.19 ± 0.25	2.35 ± 0.32	0.16	0.693
Sodium (mmol/L)	301.19 ± 7.34	312.77 ± 2.87	11.58	0.199
IFNG (pg/mL)	45.73 ± 11.30	74.86 ± 52.90	29.13	0.311
IL-1β (pg/mL)	196.61 ± 100.48	114.25 ± 45.96	−82.36	0.244
IL-6 (pg/mL)	149.15 ± 124.01	186.76 ± 80.30	37.63	0.276
C-Reactive Protein (mg/dL)	1.18 ± 0.17	0.09 ± 0.04	−1.09	**0.021***
Prolactin (ng/mL)	0.06 ± 0.01	1.32 ± 0.05	1.26	**0.000***
Cortisol (ng/mL)	4.06 ± 0.25	19.00 ± 5.51	14.94	**0.053***
Ghrelin (ng/mL)	1.08 ± 0.17	0.75 ± 0.16	−0.33	0.196
DHEA (ng/mL)	1.85 ± 0.51	5.76 ± 0.97	3.91	**0.012***

Data was analyzed using Independent Sample T Test with 95% confidence interval of the Difference

P value≤0.05 was considered significant

Table 4.5: Biomarkers Sensitive to each of the Experimental Group Using Hair Growth Pathway

Parameter	Control (Baseline)Mean ± SEM	Stress / Recovery GroupMean ± SEM	Mean Difference	P Value	Group
Sodium (mmol/L)	301.19 ± 7.34	262.45 ± 6.06	−38.74	**0.004***	Physical Stress
Calcium (mmol/L)	1.75 ± 0.02	1.65 ± 0.03	−0.10	**0.017***	Chemical Stress
C-Reactive Protein (mg/dL)	1.18 ± 0.17	0.11 ± 0.04	−1.07	**0.021***	Chemical Stress
Prolactin (ng/mL)	0.06 ± 0.01	0.12 ± 0.07	1.06	**0.003***	Chemical Stress
Ghrelin (ng/mL)	1.08 ± 0.17	0.60 ± 0.10	−0.48	**0.048***	Chemical Stress
DHEA (ng/mL)	1.85 ± 0.51	5.09 ± 3.69	3.24	**0.021***	Chemical Stress
Calcium (mmol/L)	1.75 ± 0.02	1.66 ± 0.01	−0.09	**0.005***	Chemical Stress Recovery
C-Reactive Protein (mg/dL)	1.18 ± 0.17	0.05 ± 0.03	−0.06	**0.019***	Chemical Stress Recovery
Prolactin (ng/mL)	0.06 ± 0.01	1.18 ± 0.07	1.12	**0.001***	Chemical Stress Recovery
Cortisol (ng/mL)	4.06 ± 0.25	9.41 ± 5.62	5.35	**0.016***	Chemical Stress Recovery
DHEA (ng/mL)	1.85 ± 0.51	5.73 ± 0.95	3.88	**0.011***	Chemical Stress Recovery
C-Reactive Protein (mg/dL)	1.18 ± 0.17	0.09 ± 0.04	−1.09	**0.021***	Physical Stress Recovery
Prolactin (ng/mL)	0.06 ± 0.01	1.32 ± 0.05	1.26	**0.000***	Physical Stress Recovery
Cortisol (ng/mL)	4.06 ± 0.25	19.00 ± 5.51	14.94	**0.053***	Physical Stress Recovery
DHEA (ng/mL)	1.85 ± 0.51	5.76 ± 0.97	3.91	**0.012***	Physical Stress Recovery

DISCUSSION

This study analyzed chronic stress exposure in hair using different stress biomarkers.

Calcium

This research study observed a positive association with calcium levels of chemical stress induction when compared with control(baseline) groups using hair growth pathway; this is similar with a research study carried out by Eiki, Yuichi and Fumikazoi (2013) which showed chronic stress caused a significant increase in resting ca^{2+} where mice were subjected to immobilization stress for 2 hours daily for a length of 21 days, although serum was used for the analysis.

Potassium

Potassium is the most abundant cation in the intracellular fluid and it plays a vital role in the maintenance of normal cell functions. This study observed no significant effect in potassium levels of physical and chemical stress group and also in physical and chemical recovery group compared to that control (baseline) using hair marker (P=0.830, P=0.140). This contradicts with research findings by Barbara and Nathalie,2013 which observed higher levels of hair cortisone (stress level) were associated with reduced hair levels in hair minerals ie potassium, calcium, magnesium, zinc. The findings further explained that the body's physiological stress response is related to hair minerals or mineral metabolism, such as stress – induced increased excretion of metabolites (for example cortisol and minerals) into hair. Another research study by Howee –Soo,2018 showed that potassium concentrations in hair was significant (5.05 ±4.44ug/g) in women that are fatigue and depressed and tend to decrease with increasing fatigue and depression scores (P for trend 0.037).

Sodium

Chronic stress can significantly impact sodium balance by affecting various body systems including the kidney, hormonal regulation and cardiovascular functions (Melissa *et al.,* 2022).In this study, there was significant decrease in sodium levels of physical stress when compared with the control using hair route, this showed there was reduction of stress level in sodium ion concentration using hair marker. This finding are in agreement with a research study by Howee Soo 2008 that observed Na+ concentrations in hair, a decreasing trend with increase in women with fatigue and depression scores (P=0.027 for trend) which could be classified being exposed to physical stressor. Also, this observation contradicts with the study Eleanor and Julian,2017 that observed an increased cortisol serum in response to hypo or hypernatraemic, in keeping with the stress response to illness. These research observations suggest that hair could be a sustainable biological route of assessment and detecting chronic stress using sodium as a stress biomarker.

C - reactive protein

Chronic stress is associated with elevated stress of C-reactive protein, a maker of inflammation. This suggest that prolonged stress can contribute to a state of low-grade chronic inflammation in the body. This study showed significant decrease in the CRP levels of chemical stress, chemical stress recovery and Physical stress recovery when compared with the control when using hair route. This is relative with a research study carried out by Aniruddha, 2019; data were collected from year 2006, 2010 and 2014 waves of United State Health and Retirement Study which observed that C reactive Protein positively associated with chronic stressors. This was done in both genders and in models of linear as well as non – linear change in stress levels. Also, these findings is similar to a study carried out by Amina *et al.,* 2005 on cortisol and C - reactive protein regulation in severe mental disorders which observed participant with a schizophrenia and bipolar disorder diagnosis had lower cortisol/CRP ratio (F=5.93, P=0.003) compared to the healthy controls. However, during comparison of all the experimental groups with control using serum sample there were no significant change (P > 0.05).

Prolactin

Chronic stress can lead to elevated prolactin levels, potentially disrupting normal body functions and increasing the risk of certain health disorders (Samara and Ozgul, 2018). This study observed significant increase in the prolactin level of chemical stress when compare with the control using hair route. This suggest that hair sample could be used to detect and asses chronic stress during chronic stress and after. In addition, there was significant decrease in prolactin level of chemical and physical stress when compared to their recovery period using hair route suggesting a decrease in stress level during recovery phase. With these findings observed during stress induction and recovery process, it could be said that hair is a preferred standard route when used in detecting and assessing chronic stress using prolactin as a biomarker.

Cortisol

Assessment of cortisol concentrations in hair is one of the latest innovations for measuring long term stress exposure. A research study by Gong *et al.,* 2015 found a strong correlation (r=0.6 – 0.85) between serum cortisol and corticosterone across both conditions (Both predictable and unpredictable stress). This study showed a significant effect seen in comparison of chemical stress and Physical Stress recovery with control group in hair route. This is similar to a systematic review and meta-analysis study by Ying Li *et al.,* 2023 revealed that chronic stress is associated with Hair cortisol concentration during association between chronic stress and hair cortisol in children (Pooled – r =0.09, 95% cl; 0.03, 016), Another similar epidemiologic studies by Wosu *et al.,* 2014,

where thirty- nine studies were reviewed observed to have found hair cortisol concentrations to be associated with stress related psychiatric symptoms and disorders. These findings observed during stress induction and recovery process; it could be said that hair is a preferred standard route when used in detecting and assessing chronic stress using cortisol as a biomarker.

Ghrelin

Studies has shown chronic stress significantly impacts ghrelin levels; while ghrelin is elevated in response to acute stressors, it remains chronically elevated after exposure to long term stress (Lauren *et al.,* 2020). This study observed a significant decrease in chemical stress group of hair ghrelin levels compared to the control. This is suggesting that stress levels were detected using hair route of ghrelin level.

Dehydroepiandrosterone (DHEA)

Research groups have previously described that levels of dehydroepiandrosterone are lowered in subjects who report with prolonged stress. Few studies documented lower values of DHEA-S in stressed individuals while a few reported higher values during acute stress. This study showed significant increase in the DHEA level of chemical stress in hair growth pathway when compared to the control. This findings contradicts the report carried out by Shan *et al.,* 2017 on hair measurements of DHEA ,cortisol as biomarkers of chronic stress, observed DHEA to be negatively associated to stressful life vents(P= -228,P=082).This showed high stress group has higher level hair of cortisol and lower DHEA to cortisol ratio .More also, a research study by Annalisa *et al.,* 2025 on Hair DHEA-S and cortisol ratio as long lasting biomarkers of clinical syndromes observed groups affected by enteric disease showed lower DHEA levels($P<0.0001$:1589 vs 23 pg/mg)and higher cortisol/DHEA(S) ratio ($P<0.0001$:82.83 vs 55.02) than healthy batches.

Infg

Prolonged stress can lead to the over production of stress hormone which could suppress immune function. This suppression weakens the body's ability to fend off infections increase susceptibility to illness and may contribute to development of autoimmune disorders. This study observed no significant changes in any of the four experimental groups when compared with the control using hair marker ($P>0.05$). This is in contrast with a study carried out by Eva *et al.,* 2017 that shows higher levels of INFG levels in hair after relaxation of students who went through stress during exam period.

Il-1b

Studies has showed that acute and chronic stress are associated with increased levels of proinflammatory cytokines including IL-B, IL-6, TNF–a(Margherita and Francesc Gaus ,2024).This research study showed no significant difference in IL-B concentrations of four experimental group when compared with the control using hair route. This is similar to a study by Eva *et al.,* 2017 that observed no group differences in cytokine balance (IL-1B) on hair parameters during exam period taken by medical students which concludes that in humans, stress as perceived during major medical exam can potentially shift immune response. Furthermore, comparison effect of IL-IB concentration after chemical stress and recovery phase in hair shows significant difference. This is consistent with a research study carried out on medical students during exam period and revealed that stress level; Il-IB and hair marker changed significantly from T1(Before exam period) to T2 (During the exams) but not in control.

Il-6

A study by Qing *et al.,* 2020, reported that in murine models, Psychological stress induces the release of IL-6 from adipose tissue, which acts to stimulate the fight or flight responses. This study observed no significant difference in all the experimental groups compared to the control. This observation is in contrast to a study by Arizelo *et al.,* 2019 which showed effect of induced serum IL-6 on the transcriptional signature of monocytes in circulation and brain after chronic stress. Also, another research by Elizabeth *et al.,* 2019 indicates that rodent models of restraint chronic stress at day 7 and for 14 days sequentially increased IL-6,TNF-a,C-reactive protein and acute phase reactants which demonstrates that persistent chronic stress can lead to inflammation, immunosuppression and catabolism syndrome.

CONCLUSION

Hair-growth pathway is a valuable alternate method for measuring chronic stress because it is easy, non-invasive, has a long-term perspective, provides insights into past stress experiences (Retrospective analysis) and can be

stored for extended periods of time. This research study revealed that hair growth pathway has provided a novel innovative sustainable route for the detection and assessment of chronic stress and also during recovery phase. This method rules out surgical procedure during sample collection. In other words, this study can now reliable state that using hair marker, chronic stress can be detected and assessed with female albino rats.

Contribution to knowledge

1. This work has shown that chronic stress could reliably be detected and assessed through a simple sustainable method of hair growth pathway.
2. Hair growth pathway has provided a wider research area on individual stress profiles and promoting more personalized healthcare driving advancements in the field.
3. This work has shown that chronic stress could be detected and assessed through a single and effective method of hair-growth Pathway irrespective of the origin of the stress, whether physical or chemical.

Disclaimer (Artificial intelligence)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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