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Effect Of Examination Stress On Total Leucocyte Count Of Non-Obese And Obese Medical Students

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Abstract

Objective: To assess the degree of stress before, within and after examination and to correlate it with Total Leukocyte Count (TLC), Body Mass Index (BMI) and waist-hip ratio (WHR) of participants.

Methods: 92 individuals, randomly selected, were placed into non-obese male and female (NOMs and NOFs) and obese male and female (OMs and OFs) categories, with 23 individuals in each group. The degree of stress was assessed before, during and after a crucial examination via the Perceived Stress Scale (PSS), and TLC was also assessed at each of the occasions. To compare the differences between TLC and stress, ANOVA in combination with Post Hoc Tukey's Test was used, while correlating these with BMI and WHR Pearson's correlation was applied.

Results: Obese males experienced a much higher degree of stress as compared to age-matched NOMs ($p=0.00$). Also, obese males during heightened stress showed a significant dip in their TLC as compared to NOMs ($p=0.01$) and OF ($p<0.001$). Moreover, the PSS score showed a strong negative correlation with TLC in NOMs ($r=-0.47$, $p=0.02$), NOFs ($r=-0.42$, $p=0.04$), OMs ($r=-0.73$, $p<0.001$) and OFs ($r=-0.41$, $p=0.04$).

Conclusion: Examination stress significantly affects the immune status of young adults, with obese males experiencing much more immunosuppression as compared to age and ethnicity-matched obese females as well as non-obese males.

Keywords: Leukocyte Count, Perceived Stress Scale, Body Mass Index, Waist Hip Ratio, Immunity, Obesity.

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Introduction

Stress, defined as a state that threatens homeostasis and initiates the adaptive process,[1] is categorized into two main types: short-term stress (lasting several minutes to hours and beneficial for enhancing immune function and providing resistance to diseases) and long-term stress (which persists for many hours per day over weeks or months).[2] Examination stress, characterized as a form of long-term stress,[3] is primarily induced by factors such as prolonged study hours, extended examination periods, sleep deprivation, and the competitive attitudes prevalent among students and causes long-term dysregulation of the autonomic nervous system and adrenocorticotropic hormone (ACTH) mediated release of cortisol.[4]

Stress-related long-term activation of the Sympathetic Autonomic Nervous System (SANS) mediates the release of epinephrine and norepinephrine, which, via β-adrenergic receptors activation within T helper cells and macrophages, leads to inhibition of immune cell activation.[5] Furthermore, long-term activation of SANS also causes corticotrophin-releasing hormone (CRH) mediated release of ACTH from the pituitary, which then signals the adrenals to produce cortisol. Cortisol then suppresses the production of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) while promoting the release of anti-inflammatory cytokines like interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β).[6]

Additionally, cortisol inhibits T cell proliferation by desensitizing interleukin 2-producing T cells, thereby halting the production of T cell growth factors.[7] Moreover, increased cortisol production causes inhibition of macrophage function, impairment of dendritic cell function and impairment of GALT[8], thereby exacerbating immune suppression. Obesity-associated release of pro-inflammatory cytokines like TNF-α, IL-6, and IL-1β, by stimulating the Hypothalamic-pituitary-adrenal (HPA) axis, also increases cortisol production[9]. Furthermore, obesity-related release of adipokines, i.e. Leptin and Resistin, mediates immune suppression by inhibiting the activation and proliferation of T-cells. This comes into function by inhibition of IL-2 production, a cytokine essential for T-cell proliferation and differentiation, and by increased expression of immune suppressive molecules such as PD-L1 over immune cells.[10] Hence, Obesity, like stress, also leads to considerable immune suppression and is a major global health concern and a prevalent cause of immune dysfunction.

The prime highlight of this study was to assess the extent of immunosuppression with the degree of examination stress and obesity. This study was conducted with the postulate that examination stress and obesity both lead to immunosuppression. This will help us highlight the deleterious effects of stress on the immune status of young obese adults, which in turn will help us advocate for weight management in the younger years of life.

Methodology

A prospective cohort study was conducted after ethical approval from IRB, NMU (reference no. 17820) within various medical institutes affiliated with the university. The sample size of 23 participants for each group was determined, with a power of 95% and an alpha level of 5%, using the differences in mean levels of white blood cells between the non-stressed and stressed populations of medical students[11] utilizing the formula 7.2b From the software "Sample Size Determination in Health Studies – a Practical Manual (version 2.0.21) issued by WHO Geneva":

By taking into account WHO 2000 and 2015 guidelines of obesity for the South Asian population, where individuals irrespective of gender with a BMI of >25 kg/m2 while males with a WHR of >0.9 and females with a WHR of >0.89 are considered obese[12], a potential pool of age and ethnicity matched healthy non-obese and obese medical students having ages between 18-24 years was identified through simple random sampling. The study population was then equally divided into four groups: non-obese males (NOMs - Group A), non-obese females (NOFs - Group B), obese males (OMs - Group C) and obese females (OFs - Group D) each having 23 randomly placed subjects into each group as per WHO obesity criteria described above.

Students with a previous history of psychiatric ailment, those on psycho-psychiatric and/or recreational drugs, students with a family history of stress and depression, those with ailments like endocrine disorders or exogenous hormones use, and students with genetic obesity were excluded from the study.

The degree of stress was measured using the Perceived Stress Scale (PSS) developed by Sheldon Cohen. The validity of this scale has been established with an accuracy of 78%, a positive predictive value of 28-34 ($p<0.001$), a negative predictive value of -22 ($p<0.001$), and a Cronbach's alpha of 0.754 (0.820 and 0.865 for the negative and positive subscales, respectively)[13]. It consists of 10 items measuring global perceived stress over the past 30 days on a 5-point Likert scale (0-13=mild stress, 14-26=moderate stress, 27-40=high stress).

Total leukocyte count (TLC), an indicator of immune status, was measured using the haematology analyser Sysmex (model Kx-21), which had precision (3.5% or lower for WBCs) and linearity (1.0-99.9 $\times 10^3/\mu l$ within $\pm 0.3 \times 10^3/\mu l$ or ±3%)[14].

Results:

Since the majority of our study parameters were normally distributed, our data has been represented as Mean±SD. It was noted that NOMs weighed 62.2±9.48 kg, had a height of 1.71±0.06 m, a body mass index of 21.2±2.19 kg/m^2, a waist circumference of 80.4±5.72 cm, a hip circumference of 92.7±6.59 cm and waist-hip ratio of 0.86±0.01. NOFs weighted 62.9±10.7 kg, height of 1.68±0.11 m, body mass index of 22.1±1.45 kg/m^2, a waist circumference of 73.5±3.64 cm, hip circumference of 93.3±4.86 cm and waist-hip ratio of 0.78±0.00. OMs weighted 74.6±7.11 kg, height of 1.68±0.05 m, body mass index of 26.3±1.73 kg/m^2, a waist circumference of 91.0±3.39 cm, hip circumference of 98.6±4.18 cm and waist-hip ratio of 0.91±0.01. OFs weighed 78.1±5.32 kg, were 1.74±0.05 m, had a body mass index of 25.7±1.59 kg/m^2, a waist circumference of 81.5±6.88 cm, hip circumference of 94.0±6.50 cm and waist-hip ratio of 0.86±0.02. A comparative analysis of key anthropometric indices derived from these demographics has been presented in Table 1.

After the application of ANOVA to determine the existence of differences for BMI, WHR, TLC-2 and PSS ($p<0.001$, $p<0.001$, $p<0.001$ and $p<0.001$ respectively), we through Post Hoc Tukey's Test found that stress levels during examination in NOMs and NOFs were significantly lower than OMs and OFs and that the TLC Of OMS during examination was significantly lower than in NOMs. However, there was no significant difference in TLC of NOF and OFs during the period of examination stress. These facts are detailed in Table 2 and Table 3.

It was also observed that TLC during the period of examination stress had a significant negative correlation with PSS, BMI, and WHR, indicating that stress, as well as obesity, negatively regulates the immune status of individuals. These findings are presented in Table 4. We observed that the stress levels in OMs and OFs during the examination were significantly higher than that of NOMs and NOFs, which is an observation consistent with a contemporary study[15] ($p < 0.001$). This observation of ours could be explained

by taking into consideration that biopsychosocial aspects of increased adiposity derange cortisol levels via stimulation of HPA-Axis activity[16,] which can account for higher stress within obese individuals.

Table 1: Comparison of anthropometric indices between various study groups via Post Hoc Tukey's Test (Later To ANOVA)

Variable	Groups In Comparison (n=23 for each group)		p-value
BMI	**NOMs** 21.24 ± 2.19	**NOFs** 22.12 ± 1.45	0.333
	OMs 26.31 ± 1.73	**OFs** 25.76 ± 1.59	0.721
	NOMs 21.24 ± 2.196	**OMs** 26.31 ± 1.73	<0.001*
	NOFs 22.12 ± 1.45	**OFs** 25.76 ± 1.59	<0.001*
WHR	**NOMs** 0.86± 0.01	**NOFs** 0.78 ± 0.008	<0.001*
	OMs 0.91 ± 0.01	**OFs** 0.86 ± 0.02	<0.001*
	NOMs 0.86± 0.01	**OMs** 0.91 ± 0.01	<0.001*
	NOFs 0.78 ± 0.008	**OFs** 0.86 ± 0.02	<0.001*

Table 2: Comparison of pre-, intra- and post-examination stress between various study groups via Post Hoc Tukey's Test (Later To ANOVA)

Variable	Groups In Comparison (n=23 for each group)		p-value
PSS (PeS)	**NOMs** 8.47 ± 1.78	**NOFs** 12.26±3.81	<0.001*
	OMs 10.08±3.05	**OFs** 11.17±2.38	0.573
	NOMs 8.47 ± 1.78	**OMs** 10.08±3.05	0.233
	NOFs 12.26±3.81	**OFs** 11.17±2.38	0.573
PSS (IS)	**NOMs** 20.695±4.02	**NOFs** 20.52±4.388	0.999
	OMs 26.95 ±5.44	**OFs** 29.69 ± 4.61	0.197
	NOMs 20.695±4.02	**OMs** 26.95 ±5.44	<0.001*
	NOFs 20.52 ±4.388	**OFs** 29.69 ± 4.61	<0.001*
PSS (PtS)	**NOMs** 8.82±2.99	**NOFs** 9.34±4.90	0.958
	OMs 12.21±2.98	**OFs** 11.04±2.72	0.670
	NOMs 8.82±2.99	**OMs** 12.21±2.98	0.008*
	NOFs 9.34±4.90	**OFs** 11.04±2.72	0.363

Table 3: Comparison of pre-, intra- and post-examination TLC between various study groups via Post Hoc Tukey's Test (Later To ANOVA)

Variable	Groups In Comparison (n=23 for each group)		p-value
TLC (PeS)	**NOMs** 7.07 ± 1.92	**NOFs** 7.89 ± 1.46	0.330
	OMs 7.46 ± 1.60	**OFs** 7.93±1.53	0.774
	NOMs 7.07 ± 1.92	**OMs** 7.46 ± 1.60	0.846
	NOFs 7.89 ± 1.46	**OFs** 7.93±1.53	1.000
TLC (IS)	**NOMs** 7.469 ± 1.97	**NOFs** 8.573 ± 1.61	0.130
	OMs 5.918± 1.76	**OFs** 8.036±1.38	<0.001*
	NOMs 7.469 ± 1.97	**OMs** 5.918± 1.76	0.014*
	NOFs 8.573 ± 1.61	**OFs** 8.036±1.38	0.707
TLC (PtS)	**NOMs** 7.93 ± 2.13	**NOFs** 7.62 ± 1.55	0.931
	OMs 7.15 ± 1.42	**OFs** 7.53±1.87	0.885
	NOMs 7.93 ± 2.13	**OMs** 7.15±1.42	0.439
	NOFs 7.62 ± 1.55	**OFs** 7.53±1.87	0.998

Table 4: Pearson's Correlation of anthropometric indices and stress with TLC in all the study groups

Pearson's Correlation	TLC							
	NOMs		NOFs		OMs		OFs	
	r	p	r	p	r	p	r	p
BMI	-.512	.013*	-.438	.037*	-.607	.002*	-.380	.074
WHR	-.228	.295	-.247	.255	-.020	.927	-.010	.965
PSS (IS)	-.471	.023*	-.429	.041*	-.738	.000*	-.419	.047*

Discussion

We observed that the stress levels in OMs and OFs during the examination were significantly higher than that of NOMs and NOFs, which is an observation consistent with a contemporary study[15] ($p < 0.001$). This observation of ours could be explained by taking into consideration that biopsychosocial aspects of increased adiposity derange cortisol levels via stimulation of HPA- Axis activity[16,] which can account for higher stress within obese individuals.

Furthermore, our data showed that TLC levels during the period of examination stress were significantly lower in OM than in NOMs, a fact that has been mirrored by a contemporary study as well[17]. It is also understandable if we take into consideration the immunosuppressive effects of obesity, which are primarily exerted via obesity-associated derangement of key adipocytokines like leptin adiponectin, visfatin and resistin[18], a fact which has been reported elsewhere also[19].

However, we did observe that the TLC levels of OFs and NOFs were not significantly different from each other. The plausible explanation for this could most likely be the anti-inflammatory and immunoprotective effects of estrogen in females[20]. In addition to this, it has been documented that not all genes on the silenced X chromosomes are completely silent within females, due to which they have double doses of certain genes, including TLR7, which explains why females tend to have better immune systems in general[21]. Hence, it would become plausible in the light of the above-documented facts that females, despite encountering high levels of stress as has been evident through the presented results, would be in a bio-physiological position to counter stress in a better way as compared to age and ethnicity-matched obese males.

The results of our study also depicted that though stress negatively regulated TLC in all the study groups, the suppression was more prominent within the OMs of all the groups. This once again is a finding presented by certain recent research and could be explained based on scientific ground extended by others who do state that obesity within males, via altering the testosterone levels[22], affects their immune status considerably because they do not harbour the immunoprotective genetics as are carried by their age and ethnicity matched obese females.

Our data also provides robust evidence that the TLC levels had a strong negative correlation with the BMI in NOMs, NOFs and OFs. Recent research has been projected that elevated BMI, indicative of increased adiposity, decreases the TLC levels by adipokines-mediated blockade of IL2 production[23] – an interleukin necessary for T–cell proliferation. The adiposity-induced increase in the activity of HPA–Axis further exacerbates the decrease in TLC levels by cortisol-mediated release of anti-inflammatory cytokines like IL-10 and TGF-β as is suggested by a contemporary study[24].

Though studies have projected that TLC decreases as WHR rises[25], we could not deduce such a result from our data, which revealed that though the TLC showed a negative correlation with WHR in all the study groups, it stood insignificant. This disparity of ours with the contemporary data could be explained based on different genetic make-up of south Punjabi population as compared to western ones which could have created varied responses to obesity and stress. Moreover, since our observation was based on a single-time assessment instead of serial readings and was made for a limited sample size, these two reasons might also have added to the disparity we see here.

Conclusions

Chronic stress, like that of examination, negatively affects immune status, a derangement that is profoundly seen in obese males.

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