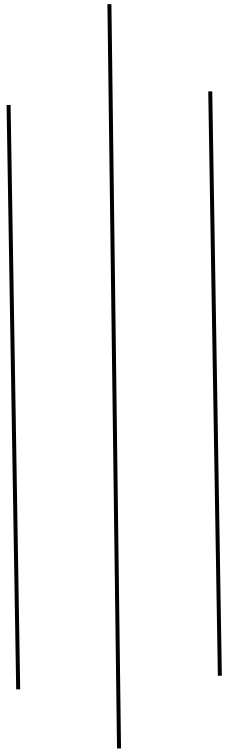
Seasonal Variation in Sympathetic Autonomic Activity in Undergraduate Health Science Students of Karnali Province



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I am also grateful to all my family, colleagues, and all those who have directly and indirectly helped me to complete this study.

Lastly, I could not remain silent without thanking all the participants of the study without whom this study could not have been generated, which can highly impact the policy makers and administrators in developing evidence-based policies and administrative responsibilities.

Sincerely,

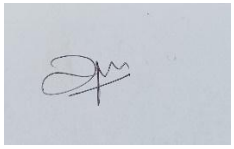

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Table of Contents

ABSTRACT

Background: Seasonal variations in autonomic function have been linked to cardiovascular morbidity and mortality. The cold pressor test (CPT) and handgrip test (HGT) have been used in various studies to determine blood pressure (BP) regulation during stress, but their differential effects in various seasons are less explored. Thus, the study aimed to investigate seasonal differences in cardiovascular sympathetic activity in healthy young adults of Nepal.

Methodology: This pre-test post-test study recruited 45 healthy students (55.6% female) aged 21.62±2.93 years, who underwent CPT and HGT interventions during summer and winter seasons. Blood pressure was measured before and after each intervention, and maximum voluntary contraction (MVC) was recorded during the handgrip test. The baseline systolic and diastolic blood pressure (SBP and DBP) and their rise during interventions were compared between summer and winter season.

Result: Baseline BP were lower in summer than in winter season (SBP: 107.71±12.17 vs 120.78±11.31, p=0.001; DBP: 71.49±8.74 vs 77.60±8.83, p=0.001; paired t-test); but the mean maximum SBP and DBP during CPT and HGT was not significantly different between seasons. In CPT, there was a statistically significant rise in BP during summer compared to winter (SBP: 25.27±11.56 vs 13.84±11.17, p<0.001; DBP: 25.53±10.14 vs 13.51±7.15, p<0.001; paired t-test). Similarly, HGT also raised the BP higher in summer than winter (SBP: 35.07±17.11 vs 25.47±14.81, p=0.008; DBP: 30.96±12.12 vs 24.16±11.64, p=0.009; paired t-test); and was confirmed in linear regression analysis (p<0.01). Hyper responders (those with >21 mmHg rise in SBP) during CPT were higher in summer (22) than winter (10).

Conclusion: This study demonstrates that while resting blood pressure is lower in summer, the sympathetic response to stress is heightened, indicating seasonal modulation of autonomic function, which may have implications for cardiovascular risk and adaptability to environmental changes.

Keywords: Autonomic, Cardiovascular health, Cold pressor test (CPT), Handgrip test (HGT), Seasonal variation, Sympathetic activity.

CHAPTER I

BACKGROUND

Our body mechanism responds to environmental changes, and annual cycles have been seen in various homeostatic parameters including autonomic function[1]. Incidence of cardiovascular diseases (CVDs), including acute myocardial infarction (MI) and cerebrovascular disease also varies with seasons[2]. There is an increased incidence of cardiovascular events in colder months. Although the exact mechanism is still on debate, one likely mechanism is that the elevation of BP is induced by over sympathetic activity as a result of exposure to cold temperatures in winter[3]. Exposure to cold temperature increases sympathetic tone and increase in noradrenaline level in blood and urine. It also causes vasoconstriction, endothelial dysfunction, activation of renin-angiotensin-aldosterone system via multiple mechanisms, which tend to increase blood pressure[4–6]. Whereas in summer, vasodilation and sweating-mediated salt and water loss tend to reduce blood pressure[4].

Stressor tests like hand immersion in cold water (4 – 10) ºC) (cold pressor test, CPT) or isometric exercise by handgrip dynamometer (handgrip test, HGT) send signal via afferent fibers like Krausse corpuscles and Ruffini organs in skin, and via afferent fibers of muscles, to reticular activation system and pressor areas in medulla. The efferent neurons cause sympathetic discharge from pressor area to heart and blood vessels which increase the SBP and DBP by 15-20 mmHg and 10-15 mmHg respectively, which stabilizes to baseline level within 5 minutes after the withdrawal of the stressor[7]. Earlier, these tests have been studied to assess integrity of sympathetic nervous system, to evaluate the difference in response due to sex and altitude, and for prediction of hypertension in future[8–10]. In case there is higher cardiovascular reactivity to a stressor and slower rate of recovery after the withdrawal of the stressor, the autonomic control system is not competent enough to lower heart rate and blood pressure to the baseline quickly, thus, increases the risk of an early onset of hypertension in the future[10]. Similarly, individuals with high baseline cardiac indices like heart rate and cardiac index, hyperresponsiveness also correlates with future development of hypertension[11]. In addition, autonomic dysregulation also attributes to the conditions such as epilepsy, cardiac failures, sleep apnoea and sudden death[12]. Studies have shown various factors influencing the

sympathetic activity including season, age, sex and body mass index (BMI). Winter-induced sympathetic activity correlates with temperature[13,14] and muscle sympathetic nerve activity is higher in winter[15]. Increasing age tends to have high blood pressure after stressor tests, which might be because of their low pronounced orthostatic reflex[4]. Males and individual with low body mass index (BMI) tends to have increased blood pressure in response to cold[6]. The cold-induced sympathetic activity is associated with rise in blood pressure in normal as well as in treated and untreated hypertensive patients[4,6]. However, the rise in blood pressure have varied by the studies with the rise in only SBP by some studies[15,16] while in both SBP and DBP by others[13,17]. Similarly, the degree of seasonal variations on blood pressure also varies with geography and race[14]. Asian people who tend to be more salt sensitive, have higher morning blood pressure surge and have higher incidence of isolated nocturnal hypertension[6]. Thus, cardiovascular sympathetic control varies with geography, meteorological factors like temperature, age, season and various disease conditions.

1.1 Need for the Study

The sympathetic response of the autonomic nervous system varies significantly across different age groups, sexes, and various pathologies. Additionally, factors such as geography, race, and seasonal temperature fluctuations can further influence these responses. While it is recognized that seasonal variations can affect various physiological processes, the specific effects on cardiovascular autonomic responses remain under explored in Nepal.

Comprehensive studies on the influence of seasonal changes on normal physiological functioning are essential, as they may provide valuable insights. Understanding these effects can assist healthcare professionals in making informed clinical decisions and developing evidence-based strategies for managing cardiovascular and autonomic function-related diseases.

Nepal, a country characterized by diverse geography and notable variations in ambient temperature throughout the seasons, could also have the impact of seasonal changes on autonomic function suggesting further investigation to enhance our understanding of normal physiology and inform clinical practice.

1.2 Objectives of the study

General Objective

- To assess the effect of seasonal variation in sympathetic autonomic activity among healthy young adult students of health science at Karnali province.

Specific Objectives

- To measure the baseline cardiovascular and stress-induced sympathetic autonomic activity in summer as well as in winter among the healthy young adult students of KAHS.
- To evaluate the rise in blood pressures during the stress tests in summer and in winter.
- To compare the baseline cardiovascular and stress-induced sympathetic autonomic activity between summer and winter among the healthy young adult students of KAHS.

1.3 Significance of the study

Climate change is believed to affect various physiological functions in the human body making it imperative to explore these impacts in specific geographical contexts. The study area is situated in the Himalayan region of Nepal, where the impact of seasonal variations on the physiology of BP has not yet been assessed. Thus, the study aims to provide descriptive evidence on sympathetic autonomic functioning during different seasons. The findings of the study have contributed to valuable insights into the effect of seasonal variation on the cardiovascular sympathetic autonomic activity in relation to seasonal variations among high-altitude populations. This information could be instrumental in developing evidence-based treatment policies and protocols for cardiovascular disease management, thereby moving away from a one-size-fits-all approach across the country. The potential implication of this research is to facilitate tailored healthcare services for those living in high-altitude areas. Additionally, the study will enrich the existing body of knowledge regarding sympathetic autonomic parameters and their seasonal fluctuations among high-

altitude residents, thereby informing healthcare stakeholders and enhancing their understanding of the unique needs of this population.

CHAPTER II

LITERATURE REVIEW

The autonomic nervous system (ANS) is the component of the peripheral nervous system that controls cardiac muscle contraction, visceral activities, and glandular functions of the body. The ANS regulates heart rate (HR), BB, respiration rate (RR), body temperature, sweating, gastrointestinal motility and secretion, as well as other visceral activities[18]. It consists of two complementary branches, the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS), which work together to maintain homeostasis[19]. The sympathetic branch is responsible for controlling the human body's reaction to situations of stress or emergency (otherwise known as the "fight-or-flight" response), while the parasympathetic is generally responsible for basal organ system function. In response to environmental and lifestyle demands, the homeostatic processes that naturally balance the sympathetic and parasympathetic nervous systems are often compromised and lead to ANS imbalance. Imbalanced ANS can result in various health issues. These can include cardiovascular problems, digestive disorders, sleep disturbances, and even mental health disorders[20].

The cardiac ANS is a crucial component in physiological and pathological responses of the cardiovascular system. Through its two branches, and effector molecules including norepinephrine and acetylcholine, the ANS orchestrates many events that allow for appropriate BP, HR, and Vaso-regulatory responses to routine daily stimuli. Dysregulation of this system due to aging, acute and chronic stress, organic and idiopathic and other causes contribute to cardiovascular pathology, including hypertension (HTN), ischemic heart disease (IHD), arrhythmias, congestive heart failure (CHF), and often contributes to fatal outcomes[21].

Epidemiologic data suggest that there are seasonal variations in the incidence of severe cardiac events with peak levels being evident in the winter[22–25]. The reasons for the seasonal variation in blood pressure are apparently complex, involving both long-term regulatory factors and acute responses to environmental temperatures[26]. The increase in daytime blood pressure from summer to winter is independently and inversely associated with indoor temperature[26], probably at least

in part because of the increase in peripheral resistance at lower environmental temperatures.

Stressor tests, such as the cold pressor test (CPT) and the handgrip test (HGT) are valuable assessments for evaluating the integrity of sympathetic cardiovascular autonomic function. These tests are designed to provoke a sympathetic response, allowing researchers and clinicians to assess how well the autonomic nervous system regulates cardiovascular responses under stress.

The CPT is a simple and non-invasive test in which the subject immerses one hand or foot into ice water for 1–3 min while BP and HR are monitored[27]. The cold stimulus activates afferent sensory pathways that, in turn, trigger a sympathetic response resulting in an increase in BP. The cold pressor test has been used in the clinical and research settings to evaluate non-baroreflex-mediated sympathetic neural control in humans[27]. If afferent or efferent neuronal circuits and pathways are damaged, the sympathetic vasomotor response is impaired and, therefore, the BP and heart rate responses to cold pressor test can vary significantly.

The HGT is a widely used simple test to assess cardiovascular autonomic functions. It provides pressor stimuli to the cardiovascular system through efferent sympathetic pathways, with a resultant increase in the HR and the BP[28]. This test underscores the importance of autonomic function in cardiovascular health and facilitates our understanding of how the ANS responds during physical stress.

In summary, the autonomic nervous system plays a foundational role in the regulation of cardiovascular health, with its two branches contributing to a delicate balance essential for homeostasis. Seasonal variations in cardiovascular events and the utility of stressor tests, such as the cold pressor test and handgrip test, might enlighten the importance of understanding the ANS's functioning and its implications for health.

CONCEPTUAL FRAMEWORK

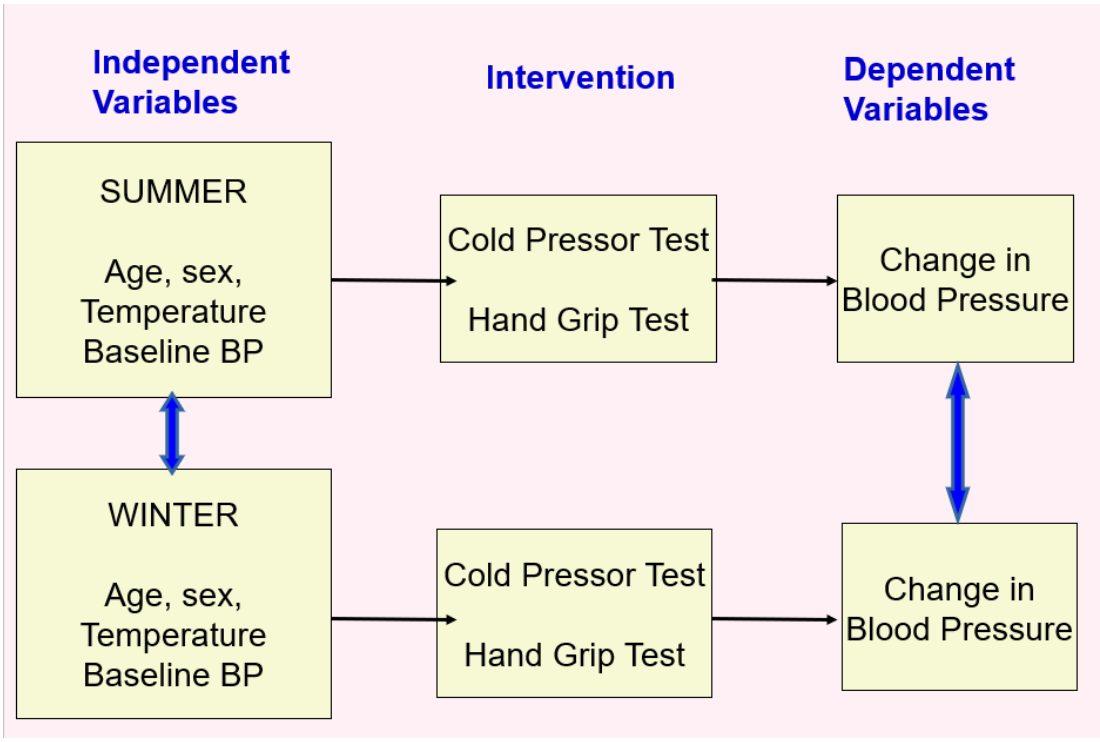

Figure 1. Conceptual framework

The conceptual framework illustrates the relationship between independent variables (Age, sex, temperature, baseline BP) and the dependent variables (Change in blood pressure) before and after the stress interventions (Cold pressure test and Hand grip test).

CHAPTER III

METHODOLOGY

3.1 Researcher characteristics

The investigators are from medical and nursing backgrounds and are currently involved in clinical, academic and research activities and research projects of medical and nursing science.

3.2 Research design

A pretest- post-test study design was applied to evaluate the effect of stressor tests (CPT and HGT) on the cardiac sympathetic activity and how it varies with seasons (winter and summer).

3.3 Study setting

The study was conducted in Autonomic Function Lab of Department of Clinical Physiology, Karnali Academy of Health Sciences, Jumla, where all the required tools and equipment are available. The main objective of the study was to assess the effect of seasonal variation on the sympathetic autonomic function among the young adults.

3.4 Study population

Study population were students of Bachelor in Midwifery Science (BMS), Bachelor's in Nursing science (BNS), Bachelor's in Pharmacy (BPharmacy) and Bachelor of Medicine & Bachelor of Surgery (MBBS) studying in Karnali academy of health sciences (KAHS) Jumla.

Inclusion Criteria

- Apparently healthy undergraduate students of Karnali Academy of Health Sciences, Jumla (KAHS) age 17-30 years willing to participate.

Exclusion Criteria

- Subjects with obvious diseases as per history and general physical examination, e.g. Hypertension, family history of cardiovascular diseases.

- Subjects under any medication that is known to alter cardiovascular or autonomic function, such as beta blockers, muscle relaxants, sedatives, tranquilizers, etc.
- Subjects who regularly take drugs of abuse.
- Subjects taking tobacco, smoke or tea/coffee within past four hours of examination.
- Subjects who cannot tolerate the test, such as those who develop Raynaud Phenomenon during cold immersion.
- Non-consenting subjects.

3.5 Sampling technique

A non-probability purposive sampling technique was used for sampling.

3.6 Sample size

Sample size was calculated using the formula: $n= 2[(Z\alpha/2 + Z_{\beta})^2 \times SD^2] / (\text{mean difference})^2$. For a mean DBP difference of 8 mm Hg and standard deviation of 13 between summer and winter (based on the study by to Kimura et al[29] taking 95% confidence and power of 80%, the minimum sample size worked out to be 41. Allowing non-response rate of 10% on follow-up visits, a minimum sample size derived was 45.

3.7 Data collection tool

- Digital Sphygnomanometer, Dr.Morepen Laboratories Ltd.
- Handgrip Dynamometer, Labycare, from SK Scientific and Surgicals
- Wall Thermometer, OMSONS Co.
- Digital weighing machine, Kinlee Co.
- Stadiometer Kinlee Co.
- Measuring tape
- Semi-structured questionnaire for demographic variables

3.8 Ethical consideration

The ethical clearance (Ref. 079/80/22) from the Institutional Review Committee (IRC) of the KAHS, Jumla and Nepal Health Research Council NHRC (Ref: 2566), was taken prior to starting the study. The purpose and details of the study, including the data collection methods and their roles and rights in the study, were explained to

the participants through the information sheet and ensured that their confidentiality will be maintained. The participants were also assured that the study will not cause any harm to the participants. The participants were also informed that they were allowed to withdraw from study at any time during the data collection process. Written consent was taken from all the participants before the data collection.

3.9 Data collection method

Each participant went through brief history taking, general physical examination and CPT and HGT during winter (December) of 2022 and in summer (July) of 2023.

Room temperature was recorded by digital room thermometer (OMSONS Co.®). Baseline information including age, sex, address, health status, course and year of study were obtained by questionnaire. Height and weight were measured with the participants dressed in light clothing and barefoot using stadiometer and weighing machine, respectively. Each parameter was measured thrice and averaged to minimize the interpersonal bias.

In the autonomic function lab, the participants were allowed to sit comfortably in a chair and explained about the test and the procedure. After about 10 minutes of rest, pulse rate (PR) at baseline was measured by palpating radial artery for 1 minute. Blood pressure was measured using digital sphygmomanometer (Dr. Morepen® Laboratories), which also measured PR during the autonomic function test (CPT and HGT). Same instrument sets were used throughout the study. After five additional minutes of rest, autonomic test was performed.

Cold Pressor Test[30]

First, the digital sphygmomanometer cuff was kept in the left hand to measure the blood pressure. Then each participant was asked to immerse their right hand, to just above the wrist, in a mixture of ice and water at 4 to 8°C temperature, for 1 minute. BP and PR were recorded at 1 minute (immediately after the test), 2 minute and 4 minutes after the test, or until the BP returns to basal level (Diastolic BP within 10 mmHg of baseline). The BP response was calculated by subtracting the baseline BP from the peak BP during and after the test. The same procedure was done in summer as well as in winter season by following up of the same subjects.

Hand grip test[31]

Each participant was asked to grip the handgrip dynamometer (Labycare®) with maximal isometric force of the dominant hand. It was repeated three times, each 2 minutes apart. The maximum value of the three readings was taken as the maximal voluntary contraction (MVC). Next, the participant was instructed to sustain the grip on the dynamometer at one third of the MVC for 4 minutes, or until fatigue. Then after, the BP and PR were measured on the contralateral arm at 1st, 2nd, 3rd and 4th minute. If a subject couldn't perform for 4 minutes fully, last BP was taken just before the release. A recovery BP was taken at 2 and 4 minutes after the release of the grip, or until the BP returns to basal level (DBP within 10 mmHg of baseline). The BP response was calculated by subtracting the baseline BP from the peak BP during and after the test.

3.10 Data analysis

All the collected data were coded and entered in a spreadsheet program. Statistical package for social sciences version 21 (IBM® SPSS® v21) were used for statistical calculation. Descriptive statistics was done to calculate frequency, percentage mean and standard deviation. Analysis was done using t-test and linear regression analysis. A p-value of <0.05 was taken as statistically significant.

CHAPTER IV

RESULT

Total 45 health sciences students participated in the study including female (n=25, 55.6%) and male (n=20, 44.4 %) with the mean age 21.62±2.93 years (range 17-30). The mean room temperature recorded were 22.5±3.35°C in summer, and 10.32±5.63°C in winter. Blood pressure before and after the intervention (CPT and HGT) in winter and summer season is illustrated in Table 1. The result shows that the baseline systolic and diastolic BP are higher in winter season compared to summer season, the difference is significant (also Figure 1). On the other hand, the maximum BP during the interventions are not different across the season except for DBP after the CPT.

Table 1. Comparison of parameters in seasons (paired t-test)

Parameters	**Winter (Mean±SD)**	**Summer (Mean±SD)**	**t-value**	**Effect Size (Cohen's d)**	**p-value**
Baseline SBP	120.78±11.31	107.71±12.16	5.85	0.87	<.001*
Baseline DBP	77.6±8.83	71.49±8.74	0.66	0.57	<.001*
Baseline Pulse Rate	4.36±5.98	7.73±10.25	3.81	-0.28	0.069
Max SBP in CPT	134.62±13.83	132.98±12.92	-3.47	0.1	0.512
Max DBP in CPT	91.11±9.88	97.02±9.15	0.93	-0.52	0.001*
Max SBP in HGT	146.24±18.66	142.78±21.03	-0.25	0.14	0.359
Max DBP in HGT	101.76±12.98	102.44±14.34	-1.86	-0.04	0.800

* Statistically significant at 99.9% confidence

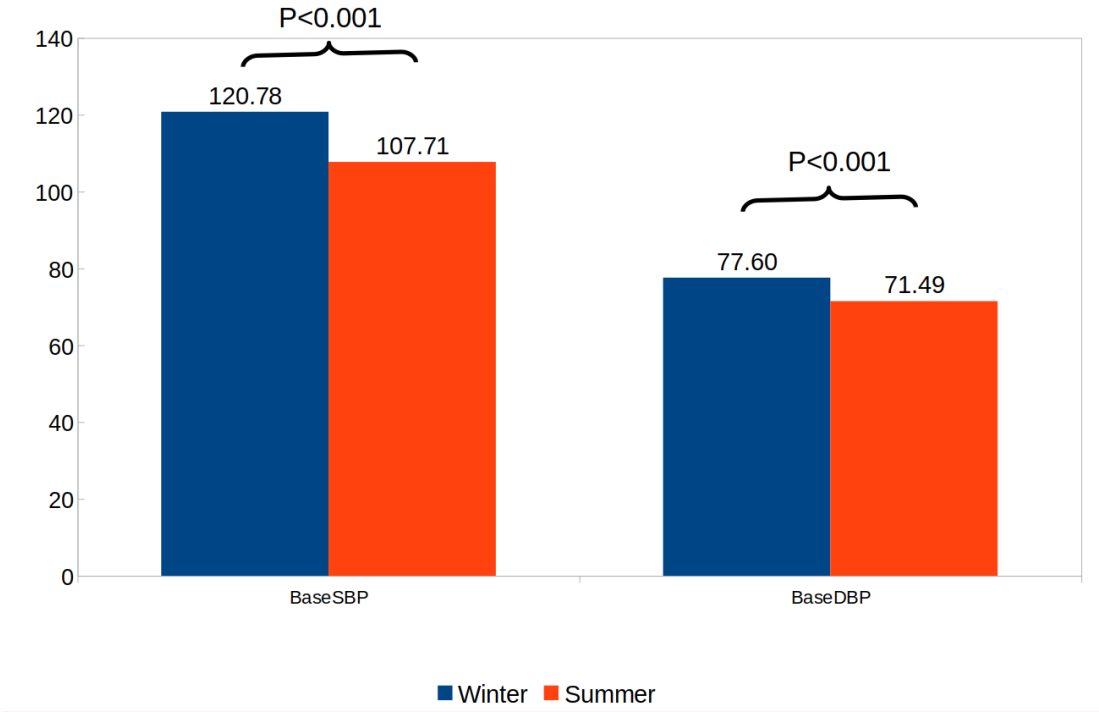

Figure 1. Baseline blood pressure comparison in the two seasons.

Following CPT and HGT intervention, there was a statistically significant rise in systolic and diastolic BP in summer compared to winter as illustrated in Table 2.

Table 2. Mean rise in SBP and DBP in Summer and Winter after CPT and HGT

Rise in Blood Pressures (Maximum - Baseline)	**In Summer (Mean±SD)**	**In Winter (Mean±SD)**	**Effect Size (Cohen's d)**	**t-value**	**p-value**
SBP during CPT	25.27±11.56	13.84±11.17	1.01	4.79	<0.001*
DBP during CPT	25.53±10.14	13.51±7.15	1.37	6.67	<0.001*
SBP during HGT	35.07±17.11	25.47±14.81	0.59	2.78	0.008*
DBP during HGT	30.96±12.12	24.16±11.64	0.57	2.74	0.009*

* Significant difference at 99% confidence.

The same comparison is graphically depicted in figure 2.

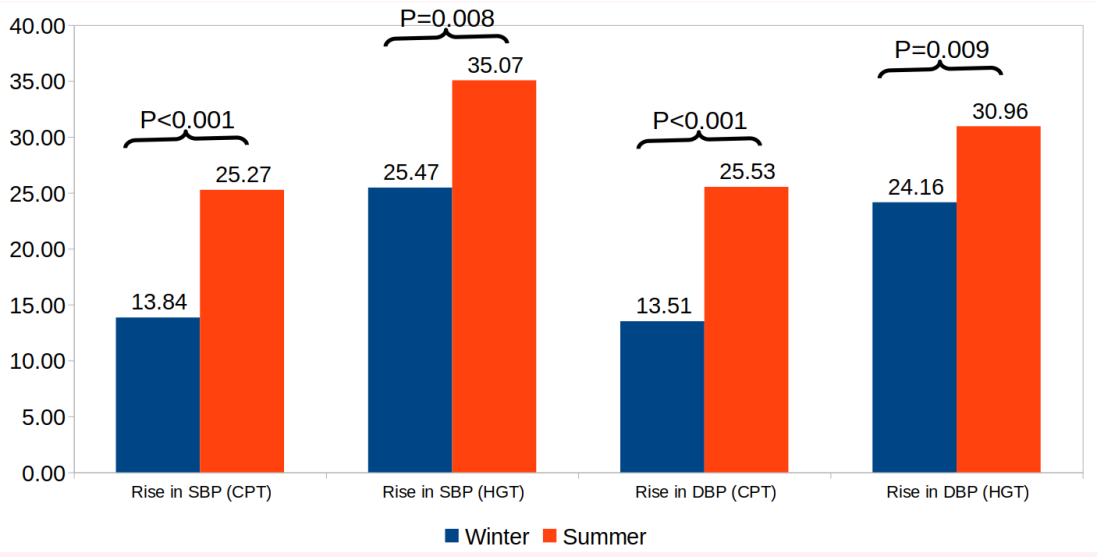

Figure 2. Rise in blood pressure during two stressor tests across the seasons.

Hyper-responders (those with >21 mmHg rise in SBP) during CPT were higher in summer (22, 48.89%) than winter (10, 22.22%). Similarly, hypo-responders (rise of DBP <10 mmHg) during CPT dropped from 15 in winter to 3 in summer; and during HGT dropped from 3 in winter to 1 in summer.

Sex comparison in the parameters is shown in Table 3. In our participants, females showed significantly higher rise in SBP during CPT in summer, but not in winter or in DBP. During HGT, males showed significantly higher rise in both SBP and DBP in winter and only in DBP in summer.

Table 3. Comparison of response to sympathetic stress test in the participants by sex (Student t-test)

Parameters	Mean (SD) Female	Mean (SD) Male	t-value	Effect Size (Cohen's d)	p-value
Baseline SBP (overall)	113.76 (13.01)	114.83 (14.0)	-0.38	-0.08	0.707
Baseline DBP (overall)	74.69 (8.33)	74.37 (10.37)	0.17	0.04	0.868
Baseline SBP in winter	119.28 (11.90)	122.65 (10.53)	-0.99	-0.30	0.326

Baseline SBP in summer	104.76 (10.53)	111.4 (13.3)	-1.87	-0.56	0.068
Baseline DBP in winter	78.4 (8.64)	76.6 (9.18)	0.68	0.20	0.503
Baseline DBP in summer	71.4 (7.22)	71.6 (10.53)	-0.08	-0.02	0.940
SBP rise in winter during CPT	16.04 (11.81)	11.1 (9.92)	1.50	0.45	0.142
SBP rise in summer during CPT	28.92 (12.35)	20.7 (8.79)	2.51	0.75	0.016*
DBP rise in winter during CPT	15.04 (6.19)	11.6 (7.95)	1.63	0.49	0.11
DBP rise in summer during CPT	26.88 (10.39)	23.85 (9.81)	1.00	0.30	0.325
SBP rise in winter during HGT	19.96 (12.15)	32.35 (15.22)	-3.04	-0.91	0.004*
SBP rise in summer during HGT	31.68 (14.16)	39.3 (19.78)	-1.51	-0.45	0.14
DBP rise in winter during HGT	20.32 (7.88)	28.95 (13.86)	-2.63	-0.79	0.012*
DBP rise in summer during HGT	26.96 (11.06)	35.95 (11.77)	-2.63	-0.79	0.012*

* Significant difference at confidence level 95%

A multiple linear regression analysis was performed to determine the effect of sex, season and the baseline BP on the SBP and DBP after the stress test intervention depicted in Table 4. It revealed that the SBP and DBP raised in the summer season after the CPT and HGT test, baseline BP has negatively influenced the rise of DBP, and sex differences were not significant in most of the cases except the SBP rise during summer season after the intervention of CPT.

Table 4. Relation of sex, season and baseline blood pressure on SBP and DBP after intervention

Independent Var (test)	Adjusted R^2	F (p-value)	Significant Predictors	Unstd Coeff (B)	Std Coeff (Beta)	P value
Rise in SBP (CPT)	0.31	8.95 (<.001)	Baseline SBP	-0.44	-0.46	<0.001**
			Season	7.62	0.3	0.004**
Rise in DBP (CPT)	0.47	16.98 (<.001)	Sex	-5.22	-0.25	0.005**
			Baseline DBP	-0.50	-0.44	<0.001**
			Season	10.61	0.50	<0.001**
Rise in SBP (HGT)	0.08	2.61 (0.030)	Season	9.41	0.28	0.017*
Rise in DBP (HGT)	0.17	4.59 (0.001)	Baseline SBP	0.31	0.33	0.016*
			Baseline DBP	-0.42	-0.32	0.014*
			Season	8.03	0.33	0.004**

* Significant at 95% confidence; ** significant at 99% confidence.

CHAPTER V

DISCUSSION

The present study aimed to investigate seasonal variations in baseline BP and sympathetic autonomic responses to the CPT and HGT in young healthy adults. Our findings revealed that baseline systolic and diastolic BP were significantly higher in winter compared to summer. Interestingly, the BP responses to both CPT and HGT were more pronounced during the summer months, which is a novel finding. These results suggest a complex interplay between environmental factors and autonomic regulation of cardiovascular function.

Previous research has established the critical role of the sympathetic nervous system (SNS) in regulating arterial BP and its alterations in various disease states, including hypertension and heart failure[32]. Our study extends these findings by demonstrating that even in healthy young individuals, seasonal factors can influence both resting BP and sympathetic reactivity.

Our findings align with previous studies demonstrating that seasonal fluctuations significantly impact blood pressure (BP) and heart rate (HR) in healthy individuals. A meta-analysis reported that both systolic and diastolic BP are higher in colder seasons compared to warmer ones, with differences averaging 3.42 mm Hg for systolic BP and 2.86 mm Hg for diastolic BP[33]. Similarly, a prospective observational study found that 24-hour BP profiles in healthy adults exhibit significant seasonal variations, with higher BP readings during winter months[34]. This seasonal modulation may have implications for understanding the environmental contributions to cardiovascular risk.

Baseline BP elevation in winter is likely driven by increased sympathetic tone for thermoregulation (such as peripheral vasoconstriction), while heightened stress reactivity in summer may reflect adaptive responses to environmental changes. Hormonal factors (cortisol, vasopressin, and other stress-related mediators) fluctuate with seasonal variations in daylight, temperature, and humidity, influencing both baseline autonomic tone and reactive sympathetic responses[36]. These findings highlight the complex interplay of environmental, metabolic, thermoregulatory,

neuroendocrine, and autonomic factors in regulating cardiovascular responses across seasons.

Studies have shown that increased sympathetic activity is a hallmark of essential hypertension[35]. Our observation suggests increased sympathetic tone in response to colder temperatures. It appears that during winter participants had a greater baseline sympathetic activity that may limit their sympathetic drive during stressors like CPT and HGT, resulting in lower pressure response in winter. Interestingly, the augmented BP response to CPT and HGT in summer suggests that, in summer, their stress-reactivity is heightened while maintaining lower sympathetic tone. A study conducted to evaluate the differences in cardiovascular and sympathetic biomarkers between responder types showed that low baseline sympathetic tone correlates to a greater blood pressure change in the cold pressor test[11]. This effect might have seen among our study participants in the summer season where the low sympathetic tone in summer might had a greater BP change in summer than winter after both the interventions. This paradoxical finding indicates that different mechanisms may underlie baseline sympathetic tone versus reactive sympathetic responses.

Gender difference in baseline BP and also in the sympathetic stress test has been reported in literature[37]. For instance, studies have reported significantly lower sympathetic nerve activity in young and middle-aged women than men suggesting women had a lower central sympathetic neural output to the periphery, as well as a lower vasoconstrictor response to this neural output[37,38]. Likewise, Ettinger et al[39] reported that cardiovascular and vasomotor sympathetic responses to handgrip test were attenuated in women. Additionally, the same group reported that increases in muscle sympathetic nerve activity (MSNA) during handgrip varied with the phase of the menstrual cycle[40]. In contrast, other suggested that women and men demonstrate comparable increases in MSNA in response to handgrip[41] and cold pressor test[42]. A study on young Indian students show that HGT generate maximum cardiovascular response and CPT is best for studying gender-related differences[43]. We have found some significant differences between male and female of our participants, but not enough to take any conclusion in this line. Further research can be designed to specifically study the sex difference, including the changes in menstrual cycle, which has not been accounted in this study.

The clinical relevance of these findings is underscored by evidence linking sympathetic overactivity to various pathologies, such as hypertension and heart failure, and by the clinical improvement by sympathetic blockade[44–46]. Understanding seasonal patterns in sympathetic activity could inform strategies for managing conditions associated with autonomic dysregulation. For instance, although hypertensive episodes might be less at rest, a heightened sympathetic reactivity in summer might necessitate closer monitoring and proactive management of individuals susceptible to stress-induced hypertensive episodes during warmer periods.

The seasonal variation of sympathetic neural activity is further demonstrated by increase in proportion of our participants with hyperresponsive CPT in summer than in winter. This seasonal shift in hyperresponsiveness could have implications for occupational health and performance, particularly in professions requiring high cognitive or physical stress, where increased sympathetic reactivity may affect performance and health outcomes.

Our current study employs both cold pressor test (CPT) and handgrip test (HGT) to assess sympathetic autonomic reactivity in different seasons, allowing proper evaluation of stress-induced cardiovascular responses in different seasons. The use of multiple regression models enables the identification of independent predictors, adjusting for potential confounders such as age, sex, and baseline BP. Furthermore, the sample consists of healthy young adults from a health sciences background, ensuring homogeneity and reducing lifestyle-related variability. These aspects together strengthen the internal validity and relevance of the findings.

Thus, this study highlights the seasonal variation in baseline blood pressure, with additional influence of season in sympathetic reactivity in stress. A greater reactivity despite lower baseline sympathetic tone in summer implies important recommendation to the young adults.

Despite this significant finding, our study has some limitations too. Seasonal variations in physical activity, dietary habits, and exposure to sunlight can affect sympathetic activity and blood pressure, which were not considered in our study. In addition, only single event of stress test was taken for the measurement during the

daytime. However, diurnal variation could be a potential confounder during the measurement although we have tried to take the measurements at around noon for all participants. This study hasn't included other markers of sympathetic activity such as cortisol or catecholamine level, which can be an important factor to understand the phenomena. Also, small sample size and single population group would limit its generalisability. Future studies might address these shortcomings, preferably making a long-term follow-up design in wider population groups which would give better conclusion about the significance of hyper responder or seasonal variability in their health.

CONCLUSION

Our findings highlight the influence of seasonal factors on both baseline BP and sympathetic reactivity in healthy young adults, with greater blood pressure reactivity observed in summer among young adults. These seasonal variations should be considered in clinical assessments and when interpreting blood pressure variability and developing strategies for managing autonomic-related health conditions. For policymakers, it advocates developing seasonally adapted health advisories. Integrating seasonal patterns into preventive healthcare strategies could enhance public health outcomes and reduce cardiovascular risk. Further research is warranted to elucidate the underlying mechanisms driving these seasonal changes and to explore their implications in different populations and clinical settings.

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APPENDIX

INFORMATION SHEET

Statement

This information sheet is about providing the details about the research titled "**Seasonal Variation in Sympathetic Autonomic Activity in Undergraduate Health Science Students of Karnali Province**" among the students of Karnali Academy of Health Sciences, Jumla.

Purpose and methods

The purpose of the study is to assess the seasonal variation in sympathetic autonomic activity in healthy young adult students of Karnali Academy of Health Science, Jumla, Nepal.

This is a pretest-posttest study on young students. Participants who are willing to participate will be assessed for the sympathetic autonomic activity through two test (cold pressure and handgrip test) in summer and winter season.

Expected duration of the participation and frequency of contact

The study will be conducted only after taking the informed written consent. The test will be conducted 2 times in summer and winter season. Each participant should provide 30 minutes for data collection.

Benefits

There is no immediate benefit, but subjects will be screened for their cardiovascular and autonomic status.

Risks

There will not be any risks in participating in this research. However, there can be a transient increase in blood pressure among some participants. If the blood pressure rises more than 120 mmHg diastolic, the procedure will be terminated, and the participants will be observed for few minutes till BP returns to baseline.

Payment/reimbursement

No payment will be provided for participation.

Voluntary participation/withdrawal

Participation in this study is free and there is no obligation to respond, you can stop at any point. No personal data will be shared with others and the information provided will be analyzed anonymously and used confidentially. Your benefits will not be withdrawn, and you will not be punished if you wish to leave the study.

Study team and the contact details

The study team includes the faculties from department of Physiology, Karnali Academy of Health Sciences, Jumla, Nepal. If you have any queries, difficulties or complaints regarding this study or if you have questions on the rights for your participation in the study or if you want to give any information regarding this study, then you can contact me in the details provided below:

Dilli Bahadur Pun

Assistant Professor, Department of Physiology

Karnali Academy of Health Sciences, Jumla, Nepal

Phone no. 9842484973

Email: dillipun173@gmail.com

Use of data

The data will be used to make a report and transmitted to concerned people and authorities through publication and presentation. The findings of the study will be evidence to develop an evidenced based health policy in future.

जानकारी फारम

बयान

यो जानकारी फारम अन्तर्गत म यस अनुसन्धानको अनुसन्धानकर्ताले यस अनुसन्धान शिर्षक : "**Seasonal Variation in Sympathetic Autonomic Activity in Undergraduate Health Science Students of Karnali Province**" को बारेमा बिस्तृत रुपमा जानकारी गराउन जादैछु। यो अनुसन्धान यस कर्णाली स्वास्थ्य बिज्ञान प्रतिष्ठान जुम्ला नेपालमा अध्यनरत बिद्यार्थीहरुमा गरिने अनुसन्धान हो।

उदेश्य

यस अनुसन्धानको उदेश्य भनेको यस कर्णाली स्वास्थ्य बिज्ञान प्रतिष्ठान जुम्ला नेपालमा अध्यनरत बिद्यार्थीहरुमा मौसमको परिवर्तन अनुसार हाम्रो शरीरको sympathetic autonomic activity को प्रतिक्रिया अध्ययन गर्न खोजिएको हो।

यो अनुसन्धान pretest-posttest अध्ययन हो। यसमा यस अनुसन्धानमा भाग लिन इच्छ्युक बिद्यार्थीहरुमा दुई प्रकारको परिक्षणबाट (Cold pressure test, Hand grip test) जाडो र गर्मि महिनामा sympathetic autonomic activity को प्रतिक्रियाको डाटा लिइनेछ ।

सहभागिताको अपेक्षित समय र अवदि

यो अनुसन्धान, सहभागीको अनुमति पछि मात्र गरिनेछ। यदि तपाई अनुसन्धानमा सहभागी हुन चाहनु हुन्छ भने, दुई पटक (जाडो र गर्मि महिना) अनुसन्धानमा सहभागी हुनु पर्नेछ। हरेक पटक तपाइको दुइवटा परिक्षणबाट sympathetic autonomic activity को प्रतिक्रियाको हेरिनेछ। हरेक सत्रमा एकजना सहभागीलाई ३० मिनेटको समय लाग्नेछ।

लाभ

यो अध्ययनमा भाग लिंदा कुनै सिधा लाभ छैन। यद्यपि, यस अध्ययनमा सहभागी हुनेहरुलाई वहाँहरुको cardiovascular र autonomic status को screening हुनेछ।

जोखिम र असुविधाहरु

यस अध्ययनमा भाग लिंदा सहभागीहरुलाई कुनै पनि जोखिम र असुविधाहरु हुने छैन, यद्दपि केहि सहभागीहरुमा क्षणिक समयकोलागि रक्तचाप बढ्न सक्छ। यदि यस परिक्षणको समयमा diastolic blood pressure १२० mmHg भन्दा माथि बढेमा, सो परिक्षण तत्काल बन्द गरिनेछ र तपाइलाई observation मा राखिनेछ।

भुक्तानी / प्रतिपूर्ति

तपाईंको सहभागिता बापत तपाईंहरुले कुनै किसिमको भुक्तानी अथवा प्रतिपुर्ती पाउनुहुने छैन।

स्वैच्छिक सहभागिता / असहभागिता

यस अध्ययनमा सहभागिता स्वैच्छिक हो र जवाफ दिन कुनै बाध्यता छैन। तपाईं कुनै पनि समयमा असहभागिता जनाउन सक्नु हुन्छ। कुनै पनि व्यक्तिगत डाटा अरूसँग साझेदारी गरिने छैन र प्रदान गरिएको जानकारीलाई गुमनाम रूपमा विश्लेषण गरिनेछ र गोप्य रूपमा प्रयोग गरिनेछ। यदि तपाईं अध्ययनबाट बाहिर जान चाहानुहुन्छ भने तपाईंको सुविधाहरू फिर्ता लिने छैन र तपाईंलाई सजाय दिइने छैन।

अध्ययन टोली र सम्पर्क विवरण

अध्ययन टोलीमा कर्णाली स्वास्थ्य बिज्ञान प्रतिष्ठानका फिजियोलोजी विभागमा कार्यरत शिक्षकहरुको सहकार्य समावेश छ। यदि तपाईंसँग यस अध्ययनको बारेमा कुनै प्रश्न, कठिनाइ वा गुनासो छ वा अध्ययनमा तपाईंको सहभागिताकोलागि अधिकार सम्बन्धी प्रश्नहरू छन् वा यदि तपाईं यस अध्ययनको बारेमा कुनै जानकारी दिन चाहनुहुन्छ भने, तपाईं तल प्रदान गरिएको विवरणमा सम्पर्क गर्न सक्नुहुनेछ।

सम्पर्क विवरण

डिल्ली बहादुर पुन

उपप्राध्यापक, फिजियोलोजी विभाग

कर्णाली स्वास्थ्य बिज्ञान प्रतिष्ठान,

जुम्ला, नेपाल

फोन नम्बर: ९८४२४८४९७३

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डाटाको प्रयोग

यस अध्ययनको डाटा, रिपोर्ट बनाउन र प्रकाशन र प्रस्तुतीकरण मार्फत सम्बन्धित व्यक्ति र निकायमा प्रस्तुत गर्न प्रयोग हुनेछ। अध्ययनको निष्कर्ष भविष्यमा एक प्रमाणित स्वास्थ्य नीति बिकास गर्न प्रयोग हुनेछ।

INFORMED CONSENT

Title: "Seasonal Variation in Sympathetic Autonomic Activity in Undergraduate Health Science Students of Karnali Province."

Namaste! I am Dilli Bahadur Pun, Assistant Professor at Department of Physiology, Karnali Academy of Health Sciences, Jumla, Nepal. I am conducting research on **"Seasonal Variation in Sympathetic Autonomic Activity in Undergraduate Health Science Students of Karnali Province"** among the health science students of Karnali Academy of Health Sciences, Jumla, Nepal. The objective of the study is to to assess the seasonal variation in sympathetic autonomic activity in healthy young adult students of Karnali Academy of Health Science, Jumla, Nepal. This is a pretest-post test study on young students. Participants who are willing to participate will be assessed for sympathetic autonomic activity through two test (cold pressure and handgrip test) in summer and winter season. The participants in the study are required to provide only 30 min of your time. I assure you that it will not harm anybody and whatever information you provide will be kept confidential. Your participation in this study is completely voluntary and you can deny participating at any time. However, I hope you will participate in this study since your participation is important. I have provided all the detailed information regarding this research in the information sheet. Please provide your written consent for the participation through this study participation declaration

Study participation declaration

I,……, have explained all the benefits and risk of this study and I am aware about it. I have been provided all the information needed to participate in this study. I have understood that I can withdraw from this study anytime I want and I am not obliged to any punishment for this and I will not be spared from not getting any benefits I will be provided. If I feel any difficulties or information regarding the study, I can contact the person whose contact details is provided in the information sheet and I provide my written consent to participate in this study.

Signature of participant …… Date:……..

सूचित सहमति

शीर्षक: **"Seasonal Variation in Sympathetic Autonomic Activity in Undergraduate Health Science Students of Karnali Province"**

नमस्ते ! म डिल्ली बहादुर पुन, कर्णाली स्वास्थ्य बिज्ञान प्रतिष्ठान, फिजियोलोजी विभागमा उपप्राध्यापकको रुपमा कार्यरत छु । माथि उल्लेखित बिषयमा म यस अस्पतालमा अनुसन्धान गर्न गैरहेको छु। यस अनुसन्धानको उद्देश्य भनेको यस कर्णाली स्वास्थ्य बिज्ञान प्रतिष्ठान जुम्ला नेपालमा अध्यनरत बिद्यार्थीहरुमा मौसमको परिवर्तन अनुसार हाम्रो शरीरको sympathetic autonomic activity को प्रतिक्रिया अध्ययन गर्न खोजिएको हो। यो अनुसन्धान pretest-posttest अध्ययन हो। यसमा यस अनुसन्धानमा भाग लिन इच्छ्युक बिद्यार्थीहरुमा दुई प्रकारको परिक्षणबाट (Cold pressure test, Hand grip test) जाडो र गर्मि महिनामा sympathetic autonomic activity को प्रतिक्रियाको डाटा लिइनेछ । यसको लागि तपाईंले ३० मिनेटको समय मात्र दिनुपर्ने हुनेछ। म तपाईंलाई आश्वासन दिन्छु कि यस अध्ययनले कसैलाई नोक्सान गर्ने छैन र तपाईले प्रदान गर्ने सबै जानकारी गोप्य राखिनेछ। यस अध्ययनमा तपाईंको सहभागिता पूर्ण रूपमा स्वैच्छिक हो र तपाईं कुनै पनि समयमा सहभागी हुन अस्वीकार गर्न सक्नुहुन्छ। यद्यपि, म आशा गर्दछु कि तपाईं यस अध्ययनमा भाग लिनुहुनेछ किनकि तपाईंको सहभागिता महत्त्वपूर्ण छ।

मैले जानकारी पानामा यस अध्ययनको बारेमा अझ बृस्तृत रुपमा जानकारी प्रदान गरेको छु। कृपया यो लिखित सहमति मार्फत तपाईंको अध्ययनको सहभागिताको घोषणा प्रदान गर्नुहोस्।

अध्ययन सहभागिता घोषणा

म .....लाई यस अध्ययनको जोखिम र फाइदाहरूको बारेमा व्याख्या गरेको छ र म यस बारे सचेत छु। यस अध्ययनमा भाग लिन मैले आवश्यक पर्ने सबै जानकारीहरू पाएको छु। मैले बुझें कि म कुनै पनि समयमा अध्ययन छोडिदिन सक्छु र म कुनै सजायको लागि बाध्य छैन वा अध्ययन छोडेमा मैले पाउने फाइदाहरूबाट बन्चित हुने छैन । यदि मलाई सहभागितामा कुनै कठिनाई भएमा, म सूचना पानामा उल्लेखित व्यक्तिलाई सम्पर्क गर्न सक्छु र म यस अध्ययनमा भाग लिनको लागि मेरो लिखित सहमति प्रदान गर्दछु।

सहभागीको हस्ताक्षर: …… मिति: …… ..

DATA COLLECTION TOOL

Pro-forma for data collection of participants

STUDY: SEASONAL VARIATION IN SYMPATHETIC AUTONOMIC ACTIVITY IN UNDERGRADUATE STUDENTS

Visit number: Room temperature:

Participant's Particulars:

Subject code: Age/sex:

Address:

Past medical history (significant): Smoking (Frequency x duration):

Any drugs currently taking (Frequency x duration): Alcohol (Frequency x duration):

Known co-morbidities: Tobacco (Frequency x duration):

Examination:

Height (m): Waist circumference (cm):

Weight (Kg): Hip circumference (cm):

Any obvious pathology (head-to-toe examination):

Cardiovascular parameters:

	Measurement	Pulse rate	Systolic BP	Diastolic BP
CPT	0 min (Resting)			
	1 min (at end of CPT)			
	2 min			
	4 min (recovery)			
HGT	0 min (resting)			
	1 min (during HGT)			
	2 min (during HGT)			
	4 min or before end of HGT			
	6 min (recovery)			

ETHICAL APPROVAL LETTER

Government of Nepal

Nepal Health Research Council (NHRC)

Estd. 1991

Ref. No.: 2566

31 March 2023

Assist. Prof. Dilli Bahadur Pun
Principal Investigator
Karnali Academy of Health Sciences
Jumla

Ref: Approval of research protocol

Dear Assist. Prof. Pun,

This is to certify that the following protocol and related documents have been reviewed and granted approval through the expedited review process for its implementation.

Protocol Registration No/ Submitted Date	142/2023 P 7 March 2023	**Sponsor Protocol No**	NA
Principal Investigator/s	Assist. Prof. Dilli Bahadur Pun	**Sponsor Institution**	NHRC Grant
Title	Seasonal Variation in Sympathetic Autonomic Activity in Undergraduate Health Science Students of Karnali Province		
Protocol Version No	NA	**Version Date**	NA
Other Documents	1. Informed consent form 2. Data collection tools 3. Work plan	**Risk Category**	Minimal risk
Co-Investigator/s	1. Mr. Jay Prakash Jha		
Study Site	Karnali Province		
Type of Review	√ Expedited ☐ Full Board **Review Date:** 31 March 2023	**Timeline of study** March 2023 to January 2024 **Duration of Approval** 31 March 2023 to March 2024 **This approval will be valid for one year**	**Frequency of continuing review** NA
Total budget of research	**NRs 1,00,000.00**		
Ethical review processing fee	**Waived as the researcher had received by NHRC Grant**		

Investigator Responsibilities

- Any amendments shall be approved from the ERB before implementing them
- Submit the support letter from the regulatory authorities in Nepal like DDA, FWD, DoHS, before implementing the

Tel: +977 1 4254220, Ramshah Path, PO Box: 7626, Kathmandu, Nepal
Website: http://www.nhrc.gov.np, E-mail: nhrc@nhrc.gov.np

KARNALI ACADEMY OF HEALTH SCIENCES
INSTITUTIONAL REVIEW COMMITTEE (IRC KAHS)
Jumla, Karnali, Nepal

Chairperson
Prof. Dr. Seshananda Sanjel

Members
Dr. Suryaman Menyangbo
Mr. Prem Prasad Panta
Dr. Kushal Bhattarai
Dr. Muna Maharjan
Ms. Monika Lama
Dr. Sandeep Shrestha

Member Secretary
Dr. Poojan K. Rokaya

Ref: 079/ 080/ 22 2 Sep 2022

Dr. Dilli Bahadur Pun
Karnali Academy of Health Sciences, Jumla, Nepal

Ref: Approval of research proposal: "Seasonal Variation in Sympathetic Autonomic Activity in Undergraduate Health Science Students of Karnali Province"

Dear Sir,

Thank you for the submission of your research proposal entitled "Seasonal Variation in Sympathetic Autonomic Activity in Undergraduate Health Science Students of Karnali Province" to the Institutional Review Committee Karnali Academy of Health Sciences (IRC KAHS) on 7th August 2022. The proposal was ethically reviewed by the IRC KAHS.

I am pleased to inform you that the above mentioned research proposal has been approved by the IRC KAHS on 2nd September 2022.

As per the IRC rules the investigator has to strictly follow the protocol stipulated in the proposal. Any change in the objective, methodology, data management and budget may be made so and implemented after prior approval from the IRC. You are requested to follow the ethical principles for health and biomedical research.

You should submit the progress of your research as per the work plan. After completion of the study you must submit a copy of the final work to the IRC KAHS.

If you have any queries, please do not hesitate to contact us.

Prof. Dr. Seshananda Sanjel
Chairperson
IRC KAHS

Email :irckahs@gmail.com, Website: www.kahs.edu.np/irc-committee

CONTRACTUAL SERVICE AGREEMENT

Contractual Service Agreement

(CSA)

An agreement made between the Nepal Health Research Council and the Contractor on 20th February, 2023

Dilli Bahadur Pun Principal Investigator (hereafter, Contractor) has been awarded by Nepal Health Research Council (NHRC) for the **Provincial Health Research Grant** of the Fiscal Year 2079/2080 entitled **"Seasonal Variation in Sympathetic Autonomic Activity in Undergraduate Health Science Students of Karnali Province"** on the terms and conditions mentioned below:

1. **Nature of the service:** The contractor should initiate the research work after the agreement with NHRC and submit final research report latest by June 13, 2023 (30th Jestha 2080).

2. **Duration of the project:** The duration of the study is six months.

3. **Payment schedule:**

   After signing the agreement -50%
   After Submission of Final Report -50%
   The Total amount: Nrs 1,00,000

4. **Deliverables:**
   - Submit two copies of the final report in hard binding copy.
   - Provide the electronic version of the final report.

5. **Income tax:**
   NHRC will deduct tax as per rule of Government of Nepal.

6. In cases where the contractor does not submit the completed project reports within the timeline agreed between the parties, the contractor is obliged to return the whole amount provided by the NHRC. If the solution is not found, NHRC reserves the right to take legal action according to applicable laws of the government of Nepal.

.....
Dr Pradip Gyanwali
Member-Secretory (Executive Chief)
NHRC

.....
Dilli Bahadur Pun
Principal Investigator