

Comparison of heart rate recovery between normotensive young adults with and without a parental history of hypertension



Satyajit Bagudai¹, Hari Prasad Upadhyay²

¹Assistant Professor, Department of Physiology, College of Medical Sciences & Teaching Hospital, Bharatpur, Chitwan, Nepal, ²Lecturer, Department of Community Medicine, College of Medical Sciences & Teaching Hospital, Bharatpur, Chitwan, Nepal

Submitted: 06-02-2020

Revised: 29-03-2020

Published: 01-05-2020

ABSTRACT

Background: Studies have reported that offspring of hypertensive parents are more likely to develop hypertension. Dysfunction of target organ starts even before the diagnosis of hypertension. Cardiac autonomic dysfunction may be the initial event in the pathogenesis of hypertension. Till now very few studies have been done to find the early outcomes in the cardiac autonomic functions in the normotensive siblings of hypertensive patients. Heart rate recovery after exercise is a useful marker for cardiac autonomic function. Since the etio-pathogenesis of hypertension is expected to affect the autonomic cardiovascular parameters even before the prehypertensive stage, the following study was carried out to analyze the heart rate recovery, in the descendent non- hypertensive young adults with and without parental history of hypertension. **Aims and Objective:** This research study was aimed to quantify and compare the difference (if any) of heart rate recovery in response to 3minute step test between non hypertensive children of non- hypertensive and hypertensive parents within an age group of 18-22 years. **Materials and Methods:** A total of 63 normotensive students were divided into one hypertensive parents(HP) group containing students with parental history of hypertension) and one non hypertensive parents group(NHP) having students without parental history of hypertension). Each student was subjected to 3minute Master step test. Recordings of heart rate were made before and after exercise. Heart rate recovery index (HRRI) of 1minute (HRR1), as well as in 2,3 and 4 minute (HRR2, HRR3, HRR4) were calculated and analyzed. **Results:** The resting (basal) heart rate as well as 1st minute heart rate recovery index (HRR1) was not significantly different in the two groups. Likewise, the 2nd minute (HRR2), 3rd minute (HRR3), and 4th minute HRR (HRR4) respectively were also not significantly different between the two groups. **Conclusion:** This study concluded that there is no significant difference in heart rate recovery among non-hypertensive young adults, with and without parental history of hypertension.

Key words: Autonomic dysfunction; Exercise; Non hypertensive young adults; 3-minute step test; Heart rate recovery index (HRRI)

INTRODUCTION

Essential hypertension is still continuing as one of the most prevalent cause of morbidity which is not only acting as a risk factor for cardiovascular mortality but also is involved in a considerable health care expenditure globally. This multi organ and multi symptomatic disease develops and progresses slowly spanning several decades

of life. Studies have reported that 25% of children with one hypertensive parent and 50% of the children with two hypertensive parents will eventually become hypertensive.^{1,2} This indicates the role of heredity playing a vital role in the development of the disease. Evidence from reported works has shown the autonomic dysfunction as a strong basis for the initiation and sustained maintenance of hypertension.³ Supportive data are there that even if

Access this article online

Website:

<http://nepjol.info/index.php/AJMS>

DOI: 10.3126/ajms.v11i3.27569

E-ISSN: 2091-0576

P-ISSN: 2467-9100

Copyright (c) 2020 Asian Journal of Medical Sciences



This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

Address for Correspondence:

Dr. Satyajit Bagudai, Assistant Professor, Department of Physiology, College of Medical Sciences & Teaching Hospital, Block-B, 3rd Floor, Bharatpur-10, Chitwan, Nepal. **Mobile No:** +977-9821241241. **E-mail:** sbagudai@gmail.com

hypertension is more common in middle aged and elderly population, prehypertension is more common in young adults especially in those who have family history of hypertension.⁴⁻⁸ Some recent studies have pointed out that an increased sympathetic and decreased parasympathetic activity in young adults alters the sympatho-vagal balance which might be the predisposing mechanism for the initiation of prehypertension.^{9,10} In spite of these recent reports, there is a meager data regarding the nature of change in autonomic balance which slowly changes the normotensive to develop into a state of pre-hypertension. Till date not much study has been reported in this part of the world to find out the stated scenario.

Heart rate recovery (HRR) is defined as the rate at which heart rate decreases within the following minutes after exercise.^{11,12} It reflects the dynamic balance and coordinated interplay between parasympathetic reactivation and sympathetic withdrawal.¹²⁻¹⁴ Abnormal heart rate recovery after exercise is an easy- to- measure tool for assessing reduced parasympathetic activity.^{15,16} Delay in the heart rate recovery after exercise has also been shown in many studies to be an independent predictor for both cardiovascular and all-cause mortality.^{17,18} With the above background, the current study was designed and executed COMS, Bharatpur to compare the HRR in young normotensive students with and without a parental history of hypertension, with an aim to understand whether any relation exist between heredity and imbalance of parasympathetic autonomic activity or not. The output of the research would highlight some authentic data regarding the cardiovascular autonomic regulation which might be helpful in finding ways for prevention of prehypertension.

MATERIALS AND METHODS

A comparative study was done in the Department of Physiology of College of Medical Sciences and Teaching Hospital, Bharatpur from October- December 2019. The study group was comprised of a total 64 students of either gender from 1st, 2nd year MBBS and BDS. The parental history of primary hypertension was obtained from the parents of the students. From parental history data, the participant students were divided into two groups. A non-hypertensive parents group (NHP) was having 41 of the students without a parental history of hypertension while another hypertensive parents group (HP) was having 23 students with a parental history of hypertension (single or both parents suffering from hypertension). The common inclusion criteria for both groups were that the students must be healthy and in the age group of 18-22yrs. They should be nonsmoker and nonalcoholic. Students having any recent or past systemic illness as well as using any

type of over the counter or prescription medication or supplements were excluded from the study. The study was done in the Human Physiology laboratory in the department of Physiology of COMS between 9AM-11AM. On the day of the study participants were instructed to report to the Exercise Physiology laboratory after a light breakfast with an abstinence from alcohol, caffeine or any type of strenuous activities for the preceding 24 hours. The participants were explained in detail about the procedure. All subjects gave written consent before the starting of procedure. The temperature of the recording room was maintained between 25-28°C. The participants were asked to void the urine before the start of the procedure. After 10 minutes of rest, age, weight, heights were taken and resting (basal) heart rate as well as blood pressure was recorded in a sitting posture. With the help of a metronome, 3-minute Master two step exercise test were conducted. The participants were instructed to come out of the exercise maneuver in the event of any type of discomfort. Heart rate was recorded just after the exercise as well as 1, 2 and 3 and 4 minutes after exercise in a sitting posture. Heart rate recovery index (HRR_I) was calculated by subtracting maximum heart rate achieved just after exercise by the heart rate measured in the 1st, 2nd, 3rd and 4th minute of post-exercise period. The data were examined and expressed as mean \pm SD. Changes in parameters within the group were analyzed using student's paired t-test for normally distributed data. Comparisons between the variables were analyzed using independent and paired t test. The null hypothesis was rejected at $p < 0.05$. SPSS version 20 and Microsoft excel were used for analysis of data.

RESULTS

The mean age of the HP group was 19.78 ± 0.90 and that of the NHP group was 19.93 ± 0.93 years. There was no significant difference in age among both the groups ($p = 0.55$). There was also no significant difference in terms of BMI (21.54 ± 4.34 and 21.28 ± 4.20 Kg/m² respectively, $p = 0.81$), and resting heart rate, (88.78 ± 13.94 and 95.66 ± 15.17 respectively, $p = 0.08$) (Table 1).

Table 1: General parameters and Basal heart rate (Using Student dependent t test)

Variable	NHP group (n=41) Mean \pm SD	HP group (n=23) Mean \pm SD	t value	P value
Age (Years)	19.93 \pm 0.93	19.78 \pm 0.90	0.61	0.55
BMI (kg/m ²)	21.28 \pm 4.20	21.54 \pm 4.34	-0.236	0.81
Resting Heart rate in beats per minute	95.66 \pm 15.17	88.78 \pm 13.94	1.790	0.08

(HRR₁=Heart rate recovery index of 1st minute, HRR₂=Heart rate recovery index of 2nd minute, HRR₃=Heart rate recovery index of 3rd minute, HRR₄=Heart rate recovery index of 4th minute)

With a successful completion of the heart rate recordings at different times after 3-minute exercise, no statistically significant difference was observed between the hypertensive parents and non- hypertensive parents group in terms of maximum heart rate achieved (154.09 ± 18.56 and 157.76 ± 29 respectively, $p=0.462$). The 1st minute HRR1 was not significantly different in the two groups (31.65 ± 9.28 and 27.51 ± 10.28 respectively, $p=0.115$). Similarly, the 2nd minute HRR2 (40.78 ± 10.25 and 39.32 ± 14.50 respectively, $p=0.67$), 3rd minute HRR3 (47.83 ± 10.43 and 42.24 ± 12.43 respectively, $p=0.073$) and 4th minute

HRR4 (49.91 ± 11.54 respectively, $p=0.294$) were also not significant as depicted in (Table 2, Figure 1 and Figure 2).

DISCUSSION

In our present study we did not find any significant difference between the resting heart rate in two groups ($p=0.08$). Even if both types of autonomic nervous system (sympathetic and parasympathetic) are involved in maintaining the resting heart rate but more so it is the predominant vagal parasympathetic tone that determines the resting heart rate in healthy resting individuals.¹⁹ With no significant difference in the age and BMI between both groups, this finding indicates that at rest, vagal parasympathetic activity between the two groups are insignificant. So at least in resting state, without disease affection, the vagal function is not altered in the study group. The maximum heart rate achieved just after the exercise is also not showing any significant difference between these two group ($p=0.46$). Maximum heart rate achieved during a physical exercise is a function of parasympathetic withdrawal and sympathetic activation.²⁰ So in this parameter also both groups are showing the same type of changes which indicates that the hypertensive parents (HP) group has no difference in parasympathetic withdrawal and sympathetic activation from the non-hypertensive parents (NHP) group. In all type of heart arte recovery index the p value was more than 0.05. It points to the fact that the recovery of heart rate after an exercise stress test is following the same pattern irrespective of the history of hereditary. In normal healthy individuals, a fast decrease in heart rate is observed in the first 30 seconds after exercise followed by a relatively slower decrease. Activation of parasympathetic nervous system is predominant in the decrease of heart rate observed in the early period after exercise whereas the withdrawal of sympathetic system is more effective in the later period.¹⁹ In both these autonomic parameters, the hypertensive parents (HP) group is not showing any difference from control group. All these findings are supporting the results of an another same type of work done by Jha et al in 2018.²¹ Contradictory findings have been reported from the work of Krishnan et al, Chinagudi et al and Pal et al which has shown an increased sympathetic and a decreased parasympathetic activity in the non- hypertensive healthy off- springs of hypertensive parents.^{10,22,23} Similar type of study done by Stolarz K has reported that the sympathetic activity was increased in the normotensive young healthy adults with a genetic history of hypertension without a change in their parasympathetic function²⁴. Whereas all the above mentioned works had heart rate variability as their tool of assessment, the works of Jha et al have taken heart rate recovery as their tool of autonomic assessment. Yamada et al and Noll et al have

Table 2: Distribution of the results of exercise test among groups

Variable (Heart rate in beats per minute)	NHP group	HP group	t value	P value
Just after exercise	157.76±19.29	154.09±18.56	0.740	0.462
HRR1	27.51±10.28	31.65±9.28	-1.599	0.115
HRR2	39.32±14.50	40.78±10.25	-0.428	0.670
HRR3	42.24±12.43	47.83±10.43	-1.823	0.073
HRR4	46.05±15.21	49.91±11.54	-1.058	0.294

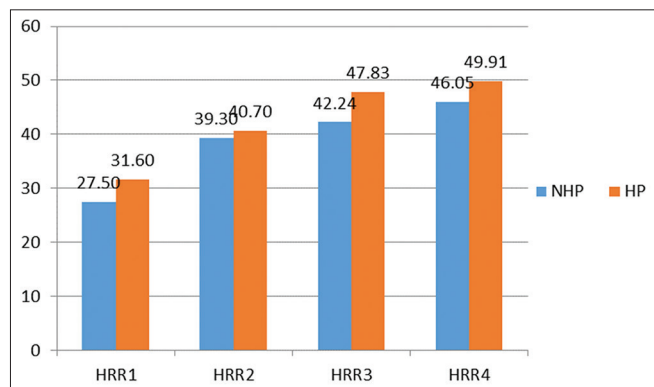


Figure 1: Bar graph showing mean heart rate recovery index in different minutes after exercise of the two groups

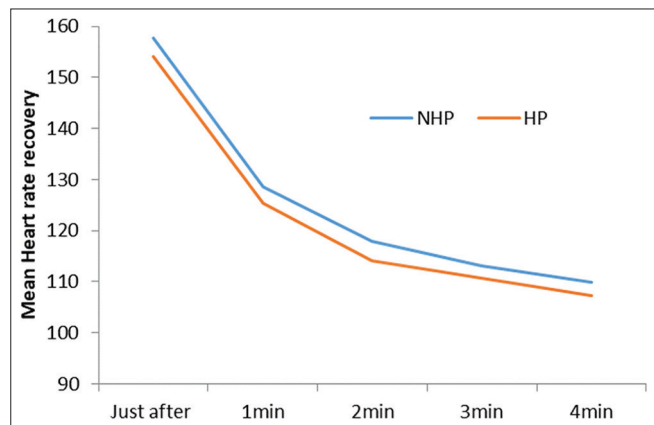


Figure 2: The Mean heart rate recovering in different minutes after exercise of both groups

measured muscle sympathetic activity using micrography in normotensive young offspring of parents with and without hypertension.^{25,26} Both studies have failed to observe any significant difference in the baseline sympathetic nerve activity between two participating groups. Similarly, Calhoun et al have reported that sympathetic reactivity to cold pressor test was not related to the parental history of hypertension in subjects of white ethnicity.²⁷

These different conflicting findings even with the works done by taking the same tool of assessment, is still posing an inconclusive consensus regarding the association between early cardiac autonomic dysfunction with genetics. Large scale prospective studies have shown an association of both maternal and paternal blood pressure with the blood pressure of their offspring.²⁸ Similarly other works have shown in their case control, cohort and longitudinal studies that parental hypertension to be strongly associated with hypertension risk.^{28,29,5} A constant advance in our understanding of blood pressure have underscored its complex pathophysiology, in which genetic and environmental factors combine for a vast number of physiological pathways and mechanisms ultimately leading to the phenotype. Till now, with many epidemiological studies giving a satisfactory understanding of the environmental factors relating to blood pressure, specifically with regard to diet and exercise, the exact contributory role of genetics has been challenging. The completion of Human Genome Project and HapMap initiatives has found a multitude of genome-wide association studies that have given the impetus to discover these genetic variants. Recent works have reported 280 genetic variants to be associated with a risk of hypertension, along with coronary artery disease for which hypertension is thought to be have a causal pathway.³⁰⁻³³ In a recent report by Warren et al a multiple of single nucleotide polymorphism (SNP) in a risk score accounted for a difference in systolic blood pressure values up to 10 mmHg between individual.³⁴ Despite this, the combined contribution of these multiple genetic variant to heritability estimates remains lower than expected.^{35,36} This has led to questions about the missing heritability.³⁷ These facts have also supported in other complex diseases like coronary disease.³⁷ Even if we expect that many more as yet undiscovered loci will help to explain about the missing heritability, now from all the works done so far, we conclude that at least in part, these contradictory reports could also be related to an overestimation of initial hereditary estimates as well as of the phenotypic pleiotropy.

CONCLUSION

There is no difference in the heart rate recovery index in 1st to 4th minute in the healthy young normotensive

adults with and without a family history of hypertension. These findings suggest that without the association of pre-hypertension and hypertension as well as other associated comorbidities, a family history of hypertension as such is not associated with a dysfunction of autonomic nervous system.

Limitations of the study

The major limitation of our present study is the less sample size of the study group. Inclusion of other confounding factors which predispose hypertension like diet, sodium intake, physical activity, age of onset of parental hypertension would have provided a better conclusion for the study. Other autonomic assessments as well as more accurate methods of sympathetic activity like measurement of plasma and urinary catecholamine metabolites should also have been compared. Therefore, more future studies with a consideration of the above facts can be done to make us reach a final conclusion regarding the association of genetics with the cardiac autonomic dysfunction even before the development of prehypertension.

Conflict of interests

The authors declare that there is no conflict of interests.

REFERENCES

- Schneider GM, Jacobs DW, Gevitz RN and O'Connor DT. Cardiovascular hemodynamic response to repeated mental stress in normotensive subjects at genetic risk of hypertension: evidence of enhanced reactivity, blunted adaptation, and delayed recovery. *Journal of human hypertension* 2003; 17: 829-840. <https://doi.org/10.1038/sj.jhh.1001624>
- Morshedi-Meibodi A, Larson MG, Levy D, O'Donnell CJ and Vasan RS. Heart rate recovery after treadmill exercise testing and risk of cardiovascular disease events (The Framingham Heart Study). *Ame J Cardiol* 2002; 90(8):848-852.
- Julius S. Autonomic nervous system dysregulation in human hypertension. *Am J Cardiol* 1991; 67: B3-B7.
- Ortega FHN, Herrera JC, Baute LH, Garc'ia AC, Gallestey JB and Ramos MR. Genetic and environmental factors in essential arterial hypertension in an urban population of Cuba. *Archivos del Instituto de Cardiolog'ia de M'exico* 1995; 65(5)426-434.
- Wang NY, Young JH, Meoni LA, Ford DE, Erlinger TP and Klag MJ. Blood pressure change and risk of hypertension associated with parental hypertension: The Johns Hopkins precursors study. *Archives of Internal Medicine* 2008; 168(6): 643-648. <https://doi.org/10.1001/archinte.168.6.643>
- Lopes HF, Silva HB and Consolim-Colombo FM. Autonomical abnormalities demonstrable in young normotensive subjects who are children of hypertensive parents. *Brazilian Journal of Medical and Biological Research* 2000; 33(1):51-54. <https://doi.org/10.1590/S0100-879X2000000100007>
- deVisser DC, VanHooft IMS, VanDoornen JP, Hofman A, Orlebeke JF and Grobbee DE. Cardiovascular response to physical stress in offspring of hypertensive parents: Dutch hypertension and offspring study. *Journal of Human Hypertension* 1996; 10(12):781-788.

8. Sowmya R, Maruthy KN, and Gupta R. Cardiovascular autonomic responses to whole body isotonic exercise in normotensive healthy young adult males with parental history of hypertension. *Indian Journal of Physiology and Pharmacology* 2010; 54(1):37–44.
9. Pal GK, Amudharaj D and Pal P. Study of sympatho-vagal imbalance by spectral analysis of heart rate variability in young pre-hypertensives. *Indian Journal of Physiology and Pharmacology* 2011;55(4) 61–67.
10. Muralikrishnan K, Balasubramanian K and Rao BV. Heart Rate Variability In Normotensive Subjects With Family History of Hypertension. *Indian J Physiol Pharmacol* 2011;55(3):253-261.
11. Cole CR, Blackstone EH, Pashkow FJ, Snader CE and Lauer MS. Heart-rate recovery immediately after exercise as a predictor of mortality. *N Engl J Med* 1999; 341:1351–1357. <https://doi.org/10.1056/NEJM199910283411804>
12. Pecanha T, Silva-Junior ND and Forjaz CL. Heart rate recovery: autonomic determinants, methods of assessment and association with mortality and cardiovascular diseases. *Clin Physiol Funct Imaging* 2014;34:327–339. <https://doi.org/10.1111/cpf.12102>
13. Coote JH. Recovery of heart rate following intense dynamic exercise. *Exp Physiol* 2010; 95:431–440. <https://doi.org/10.1113/expphysiol.2009.047548>
14. Borresen J and Lambert ML. Autonomic control of heart rate during and after exercise: measurements and implications for monitoring training status. *Sports Med* 2008; 38:633–646. <https://doi.org/10.2165/00007256-200838080-00002>
15. Imai K, Sato H, Hori M, Kusuoka H, Ozaki H, Yokoyama H, et al. Vagally mediated heart rate recovery after exercise is accelerated in athletes but blunted in patients with chronic heart failure. *J Am Coll Cardiol* 1994;24:1529-1535.
16. Arai Y, Saul JP, Albrecht P, Hartley LH, Lilly LS, Cohen RJ, et al. Modulation of cardiac autonomic activity during and immediately after exercise. *Am J Physiol* 1989;256(1)H132-141. <https://doi.org/10.1152/ajpheart.1989.256.1.H132>
17. Ho JS, Fitzergard SJ, Barlow CE, Cannaday JJ, Kohl HW, Haskell WL, et al. Risk of mortality increases with increasing number of abnormal ST parameters recorded during exercise testing. *Eur J Cardiovasc Prev Rehabil* 2010;17:462-468. <https://doi.org/10.1097/HJR.0b013e328336a10d>
18. Savonen KP, Kiviniemi V, Laaksonen DE, Lakka TA, Laukkanen JA, Tuomainen TP, et al. Two minute heart rate recovery after cycle ergometer exercise and all cause mortality in middle aged men. *J Intern Med* 2011;270:589–596. <https://doi.org/10.1111/j.1365-2796.2011.02434.x>
19. Koeppen BM and Stanton BA. Integrated control of cardiovascular system. *Bern and Levy Physiology*, 6e (Updated edition). Mosby(Elsevier). Missouri 2012:393-414.
20. Poehling PC and Llewellyn TL. The effects of submaximal and maximal exercise on heart arte variability. *Int J Exerc Sci* 2019;12(2):9-14.
21. Jha A, Karki P, Agrahari R and Kumari N. Effect of exercise on heart arte recovery index in normotensive offspring with family history of hypertension. *Int J Res Med Sci* 2018;6(4):1101-1103. <https://doi.org/10.18203/2320-6012.ijrms20181263>
22. Chinagudi S, Herur A, Patil S, Sasikala GV and Roopa A. Comparative study of Heart rate variability in normotensive offsprings of hypertensive parents. *Biomedical Research* 24(1): 123-126.
23. Pal GK, Pal P, Nanda N, Lalita V, Datta TK and Adithan C. Sympathovagal Imbalance in Prehypertensive Offspring's of Two Parents versus One Parent Hypertensive. *International Journal of Hypertension* 2011. <https://doi.org/10.4061/2011/263170>
24. Stolarz K, Grodzicki T, Lubaszewski W, Adamkiewicz-Piejko A, Olszanecka A and Kawecka-Jaszcz K. Heart rate variability in offspring of hypertensive parents. *Przegl Lek* 2002; 59:892–894.
25. Yamada Y, Miyajima E, Tochikubo O, Matsukawa T, Shionoiri H, Ishii M, et al. Impaired baroreflex changes in muscle sympathetic nerve activity in adolescents who have a family history of essential hypertension. *J hypertension. Supplement: official J Inter Soci Hypertension* 1988;6(4):S525-S528. <https://doi.org/10.1097/00004872-198812040-00165>
26. Noll G, Wenzel RR, Sheinder M, Oesch V, Binggelic C, Shaw S, et al. Increased activation of sympathetic nervous system and endothelin by mental stress in normotensive offspring of hypertensive parents. *Circulation* 1996;93(5):886-889. <https://doi.org/10.1161/01.CIR.93.5.866>
27. Calhoun DA and Mutinga ML. Race, family history of hypertension, and sympathetic response to cold pressor testing. *Blood pressure* 1997;6(4):209-213. <https://doi.org/10.3109/08037059709062071>
28. Havlik RJ, Garrison RJ, Feinleib M, Kannel WB, Castelli WP and McNamara PM. Blood pressure aggregation in families. *Am J Epidemiol* 1979;110(3):304-312. <https://doi.org/10.1093/oxfordjournals.aje.a112815>
29. Higgins MW, Keller JB, Metzner HL, Moore FE and Ostrander LD. Studies of blood pressure in Tecumseh, Michigan, II: antecedents in childhood of high blood pressure in young adults. *Hypertension* 1980;2(4):117-123. <https://doi.org/10.1161/01.HYP.2.4.117>
30. Friedman GD, Selby JV, Quesenberry CP, Armstrong MA and Klatsky AL. Precursors of essential hypertension: body weight, alcohol and salt use, and parental history of hypertension. *Prev Med* 1988;17(4):387-402.
31. Thomas CB and Duszynski KR. Blood pressure levels in young adulthood as predictors of hypertension and the fate of the cold pressor test. *Johns Hopkins Med J* 1982;151(3):93-100.
32. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007; 447:661–678. <https://doi.org/10.1038/nature05911>
33. Ehret GB, Chasman DI, Jackson AU, Schmidt EM, Johnson T, Thorleifsson G, et al. The genetics of blood pressure regulation and its target organs from association studies in 342,415 individuals. *Nat Genet* 2016; 48:1171–1184. <https://doi.org/10.1038/ng.3667>
34. Warren HR, Evangelou E, Cabrera CP, Gao H, Ren M, Mifsud B, et al. Genome-wide association analysis identifies novel blood pressure loci and offers biological insights into cardiovascular risk. *Nat Genet* 2017; 49:403–415. <https://doi.org/10.1038/ng.3768>
35. Hoffmann TJ, Ehret GB, Nandakumar P, Ranatunga D, Schaefer C, Kwok PY, et al. Genome-wide association analyses using electronic health records identify new loci influencing blood pressure variation. *Nat Genet* 2017; 49:54–64. <https://doi.org/10.1038/ng.3715>
36. Patel RS, Masi S and Taddi S. Understanding the role of genetics in hypertension. *European Heart Journal* 2017; 38:2309-2312. <https://doi.org/10.1093/eurheartj/ehx273>
37. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorf LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. *Nature*;2009; 461:747–753. <https://doi.org/10.1038/nature08494>

Authors Contribution:

SB- Concept and design of the study, collected data and review of literature, manuscript preparation, critical revision of the manuscript and preparing first draft of manuscript; **HPU-** statistically analyzed and interpreted, review of study.

Work attributed to:

Department of Physiology, College of Medical Sciences & Teaching Hospital, Bharatpur, Chitwan, Nepal.

Orcid ID:

Dr. Satyajit Bagudai -  <http://orcid.org/0000-0002-6165-9314>

Source of Support: None, **Conflict of Interest:** None.