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Autonomic and Vascular Function in Cardiovascular Health with Insights from COVID-19

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Burden of cardiovascular diseases: global and local situation

Cardiovascular diseases, which include ischemic heart disease and cerebrovascular events, have been responsible for the highest number of deaths globally, for the past several decades (1). The situation in Sri Lanka is not much different, where the number one killer and number one cause of morbidity is ischemic heart disease (1). Therefore, in the local context and the global context, it is important that risk factors and mechanisms contributing to cardiovascular diseases are identified early and interventions are implemented.

There are many modifiable and non-modifiable risk factors for cardiovascular diseases. Interestingly, two initial derangements in physiology thought to be common to most of these risk factors are autonomic dysfunction and vascular dysfunction. To understand how autonomic and vascular dysfunction could lead to cardiovascular diseases, let us first see what is meant by autonomic function and vascular function, and their relation to cardiovascular health.

Autonomic function in relation to cardiovascular health

The autonomic nervous system regulates essentially all body functions. Regulation of heart and blood vessel function via the autonomic nervous system and arterial baroreflex involves multiple interrelated control mechanisms, that through alterations in heart rate, cardiac contractility, and vascular tone, ultimately regulate blood pressure and tissue perfusion (2). Therefore, it is not difficult to comprehend that arterial baroreflex dysfunction or autonomic nervous system dysregulation would lead to alterations in cardiac and vascular function. Baroreceptor dysregulation and autonomic nervous system dysregulation are increasingly implicated in the pathogenesis of cardiovascular diseases (2). Indeed, there is overwhelming evidence that sympathetic activity is elevated, and baroreceptor function is altered not only in well-established cardiovascular diseases (3–6), but also in cardiovascular risk populations such as those with a family history of hypertension and cardiovascular diseases, obesity, diabetes, and chronic kidney disease (7–9).

Assessment of cardiac autonomic function

Several non-invasive techniques have been developed to assess the integrity of the autonomic nervous system, with respect to its effects on the heart and the blood vessels. These techniques provide us with critical information on autonomic modulation of cardiovascular function. Some of the work that is presented in this oration will

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include the assessment of specifically cardiac autonomic functions. One such measure is heart rate variability which measures the fluctuation in the time intervals between adjacent heartbeats, and therefore provides information on autonomic modulation of heart rate (10). Heart rate variability is quantified using several standard measures of sympathetic and parasympathetic function that are calculated from a continuous ECG recording (11).

A second measure of cardiac autonomic function is cardiac baroreflex sensitivity which quantifies the baroreflex activity on the sino-atrial node or in other words change in heart rate to fluctuations in blood pressure (12). This is assessed as the slope of the relationship between change in R-R interval to changes in systolic blood pressure for three consecutive beats and is termed baroreflex gain or sensitivity (13). Spontaneous baroreflex gain refers to the change in R-R interval to spontaneous changes in beat-to-beat blood pressure and is calculated from a continuous recording of heart rate and beat-to-beat blood pressure obtained during quiet rest (13).

Cardiac autonomic function is also measured using a battery of established autonomic tests known as cardiac autonomic reflex tests (CARTs) (14). This includes a head up tilt test or lying to standing test to assess the heart rate and blood pressure response to orthostasis, deep breathing test to assess the autonomic control of respiratory sinus arrhythmia, Valsalva maneuver in which a forced expiration is performed at a fixed pressure for a fixed duration to assess baroreflex function and autonomic control of heart rate, and isometric handgrip test to assess sympathetic control of blood pressure. Collectively these four tests are used to diagnose cardiac autonomic neuropathy, and all the measures described thus far provide important information on cardiac autonomic function.

Vascular function in relation to cardiovascular health

Similar to autonomic function, vascular function is also an important determinant of cardiovascular health (15). Although blood vessels have many functions, maintenance of vascular tone via an intricate balance between vasoconstriction and vasodilation is considered one of the most important functions of blood vessels. Therefore, vascular dysfunction often refers to a derangement in regulation of vascular tone that usually results in impaired vasodilation, which frequently occurs due to an impairment in nitric oxide mediated dilation. Impaired vascular function is thought to be one of the first derangements in homeostasis that results in atherosclerosis and cardiovascular diseases (15). In support of this theory, there is overwhelming evidence of vascular dysfunction in populations at risk of cardiovascular diseases such as individuals with obesity and insulin resistance (16), dyslipidemia (17), and hypertension (18,19).

Assessment of peripheral vascular function

Importantly, vascular dysfunction is reversible by lifestyle modifications and medication, and vascular function can be assessed non-invasively in different vascular beds. One such site is the upper limb, which provides information on peripheral vascular function. Peripheral vascular function is frequently assessed using a technique known as brachial artery flow-mediated dilation where the dilation of the brachial artery following a period of forearm ischemia is measured using duplex Doppler ultrasound (20). Forearm ischemia is induced by rapidly inflating a cuff placed on the forearm to a suprasytolic pressure for five minutes. The shear-mediated dilation in the brachial artery that occurs after the cuff is released, is flow-mediated dilation or FMD and represents macrovascular endothelial vasodilator function. This dilation in the brachial artery is primarily mediated by endothelial nitric oxide (21). Using the same technique, microvascular function can also be measured by quantifying the increase in blood velocity in the brachial artery after the cuff is released, which occurs due to reactive hyperemia. A higher FMD and peak blood velocity represents better macrovascular and microvascular function respectively, and importantly, lower FMD (22) and peak velocity are associated with greater cardiovascular disease risk (23).

Similar to peripheral vascular function, cerebral vascular function can also be assessed using non-invasive techniques. One technique that is used to

assess cerebral vascular function is known as cerebral vasomotor reactivity to hypercapnia (24). In this technique, the middle cerebral artery blood velocity and beat-to-beat blood pressure are measured using transcranial Doppler ultrasonography and finger photoplethysmography respectively, while the participant breathes expired air through a rebreathing bag for a predetermined period. The ensuing hypercapnia that occurs due to inhalation of expired air causes dilation of the small cerebral arterioles, which results in an increase in the blood velocity in the upstream middle cerebral artery (25). The increase in middle cerebral artery blood velocity and the cerebral vascular conductance are measures of cerebral vasodilator function, and a greater increase in these two indices indicates better cerebral vasodilator function (25).

Another aspect of vascular health that is relevant to cardiovascular health is arterial stiffness. Central arterial stiffness is non-invasively measured as carotid-femoral pulse wave velocity (PWV) which measures the time taken for the pulse wave to travel the distance from the carotid artery to the femoral artery. Carotid-femoral PWV is measured by simultaneously recording the carotid pulse and femoral pulse and calculating the velocity of the pulse wave (26). Importantly, a higher PWV indicates higher arterial stiffness, which has been shown to be predictive of hypertension and future risk of cardiovascular events (27).

My interest in vascular and autonomic physiology was ignited after I attended a workshop on Physiological Techniques at the All-India Institute of Medical Sciences, New Delhi in 2015. Following this exposure, I realized my passion in this area and decided to shape my research career towards investigating vascular and autonomic health in patients and populations at risk of developing cardiovascular disease. Thus, from here onwards I will present some of the work I have done over the past few years in this area. This includes research carried out during my postgraduate studies on Coronavirus disease 2019 (COVID-19) and vascular and autonomic health, and work that I am doing at the autonomic function testing laboratory at the Department of Physiology, Faculty of Medicine, University of Peradeniya.

COVID-19 and Vascular and Autonomic Health

Now to shift your focus to COVID-19, the disease caused by the (severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus, it was first reported among humans in December 2019 in Wuhan, China28 and declared a global pandemic by March of 2020 (29).To date, it is estimated that over 80% of the global population has been infected by the virus at least once. And although not routinely tested in Sri Lanka, it is evident from data from other countries that COVID-19 surges are still happening worldwide, and unfortunately, it is also predicted to become an endemic disease (30).

COVID-19 was initially classified as a respiratory illness. However, before long, it became clear that this assumption was not entirely accurate. For example, observations from hospitalized patients and autopsy studies during the early phase of the pandemic revealed that COVID-19 is in fact a systemic illness affecting many organ systems. Notably, cardiovascular complications and cerebrovascular complications have been frequently observed during the acute phase of the illness not only in those with comorbidities and older adults but surprisingly also in previously healthy young adults (31–38). These observations led scientists to question whether COVID-19 might in fact be a primary disease of the vasculature. However, since COVID-19 is due to a novel virus, there was not much research in this regard and minimal evidence regarding potential persistent health effects of COVID-19 on vascular and autonomic function in survivors. Therefore, based on the above observations, my research was aimed at investigating the short-term and long-term impact of COVID-19 on vascular and autonomic function with a focus in young adults. Notably, although COVID-19 affects all age groups, young adults account for a substantially higher number of COVID-19 cases compared to all other age groups including older adults (39), and studies on this age group were lacking. Therefore, my initial research focused on this age group.

In January of 2021, one study reported that peripheral vascular function was significantly blunted, and central arterial stiffness was elevated in young adults who had COVID-19 compared to those who had never had COVID-19 (40).This was a very surprising finding since the individuals in

that study were young otherwise healthy adults who had only mild COVID-19-related symptoms and no other apparent cardiovascular complications. However, these findings were from individuals who were within four weeks of COVID-19 diagnosis or in other words the acute phase of the illness, a time when the acute inflammatory effect of the virus is likely to be affecting vascular function. Around the time that these data were published, there were reports that some individuals, including those who had only mild acute COVID-19, were experiencing symptoms and complications from COVID-19 well beyond the acute illness. Thus, in our first study, we aimed to investigate whether the observed impairments in peripheral vascular function and arterial stiffness in young healthy adults persist beyond the acute phase. In addition, we also investigated whether cerebral vascular function is affected in young adults beyond the acute phase of the illness given that cerebrovascular events and cognitive issues were reported to be present following COVID-19.

Young, otherwise healthy adults who were at least four weeks past their COVID-19 diagnosis and age-matched individuals who had not had COVID-19 were recruited, and peripheral vascular function, central arterial stiffness, and cerebral vascular function were assessed using the techniques explained previously. Venous blood for inflammatory markers, and information on their COVID-19 symptomology were also obtained. Interestingly, our initial results indicated that there was no difference in peripheral macro and microvascular function, cerebral vascular function, and central arterial stiffness between young adults who had COVID-19 and were beyond the acute phase of the illness and those with no history of COVID-19 (41). However, when we then looked at our participants information more carefully, we realized that half of our participants in the COVID-19 group had persistent symptoms at the time we tested them while in half of them, symptoms had resolved. Then we questioned whether there could be a difference in vascular function measures between those with and without persistent symptoms. When the data was reanalyzed after separating the group by whether they had persistent symptoms or not, we observed that both FMD and peak velocity are blunted in young adults who were still symptomatic beyond the acute phase of COVID-19. In those whose symptoms had resolved, vascular function was comparable to the control group. This suggests that peripheral vascular function is blunted only in young adults still symptomatic beyond the acute phase of COVID-19. Regarding cerebral vascular function, although measures of cerebral vasodilator function increased with increasing hypercapnia as expected in all three groups, there was no difference between the groups indicating that cerebral vascular function was not affected beyond the acute phase regardless of symptomology. Likewise, central arterial stiffness was also not different between groups (41). Lastly, we looked at high sensitivity C-reactive protein (hsCRP) as a marker of inflammation as well as cardiovascular risk marker. We found that hsCRP was higher in those with persistent symptoms compared to those without symptoms at the time of testing, thus suggesting that inflammation could potentially be a link between persistent symptoms and peripheral vascular dysfunction (42).

The focus of my next study was on blood pressure. My initial question was whether COVID-19 had an impact on blood pressure in young adults. The literature available at that point was not conclusive, as some reported a high blood pressure of (40) while others reported normal blood pressure (43) following COVID-19 in this population. We hypothesized that one potential cause for this discrepancy could be that the time since COVID-19 diagnosis of the study participants varied greatly between and within these studies, where in some studies participants were in the acute phase while in some of them participants were up to one year out from the initial diagnosis. In addition, blood pressure was only measured in the laboratory setting. Therefore, in our next study, we aimed to perform a comprehensive assessment of blood pressure using laboratory as well as ambulatory measurements in young adults who had COVID-19 (44). A cross-sectional study was carried out where we studied young adults who had COVID-19 and were within a range of 3 to 22 weeks from diagnosis, and a control group who had not had COVID-19. Intermittent automated blood pressure and beat-to-beat blood pressure were measured in the lab setting, and 24-hour ambulatory blood pressure was also measured for better assessment of overall blood pressure status during both day and night. The results of this study revealed that blood pressure was not different

between those who had COVID-19 and controls irrespective of whether it was daytime or nighttime blood pressure. However, in the COVID-19 group, there was an inverse relationship between time since COVID-19 diagnosis and ambulatory blood pressure during daytime and nighttime, indicating that those who were studied closer to their COVID-19 diagnosis had greater blood pressure than those who were further away from the time of diagnosis. This finding was accompanied by the observation that central arterial stiffness was also independently and inversely correlated with time since COVID-19 diagnosis. Collectively, these findings indicate that negative effects of COVID-19 on blood pressure and arterial stiffness are transient and may resolve over time in young adults (44).

The studies I have described so far provide evidence for vascular dysfunction following COVID-19. There were also early reports of autonomic dysfunction following COVID-19 especially in older adults and patient populations (45,46). However, in young adults, the results were again conflicting (47,48). Given our previous findings of the influence of persistent symptoms and time since diagnosis on the impact of COVID-19 on vascular health, in the next study we aimed to investigate the impact of COVID-19 on autonomic function in young adults with and without persistent symptoms, and to determine whether there is a relationship between autonomic function and time since COVID-19 diagnosis in this population (49). For this, we recorded resting heart rate and beat-to-beat blood pressure continuously for 10 minutes in young adults who had COVID-19 and were symptomatic, those whose symptoms had resolved, and controls who never had COVID-19. Standard measures of heart rate variability and spontaneous cardiac baroreflex sensitivity were calculated from this data. Findings of this study indicated that there was no difference in measures of heart rate variability and spontaneous cardiac baroreflex sensitivity between young adults with symptomatic COVID-19, those who were asymptomatic, and controls. However, we did observe a relationship between measures of cardiac autonomic function and time since COVID-19 diagnosis whereby there was a transient negative effect of COVID-19 on cardiac autonomic regulation evidenced by lower parasympathetic function and lower baroreflex sensitivity in those closer to diagnosis compared to those further out from diagnosis. In conclusion, these findings suggest that similar to blood pressure, the effect of COVID-19 on measures of cardiac autonomic function appears to be transient and independent of persistent symptoms in young adults (49).

During the course of the pandemic, many variants of the SARS-CoV-2 virus emerged with the earlier variants such as the alpha and delta variants causing more severe illness and complications compared to latter variants such as the omicron variant. Thus, it was apparent that the outcome of COVID-19 was changing. In addition, immunity caused by the rising number of individuals vaccinated against SARS-CoV-2 also reduced the severity of disease. All our previous studies were carried out during the time the alpha and delta variants were the prominent circulating variants and vaccines were not freely available. Therefore, given the changing characteristics of the disease, in the next study we aimed to characterize whether recent breakthrough illness with COVID-19 during the omicron wave impacted vascular health and autonomic function in young adults who were vaccinated against COVID-19 (50). Peripheral vascular function, central arterial stiffness, and measures of cardiac autonomic function were assessed in young adults who had been vaccinated but contracted the infection during the omicron wave, and age-matched vaccinated controls who had never had COVID-19. Our findings from this study were quite encouraging in that measures of vascular health, and cardiac autonomic function were not different between the two groups (50). Given that previous data showed evidence of vascular and autonomic dysfunction following infection with the earlier variants, these data demonstrate that SARS-CoV-2 variants may have differential effects on vascular and autonomic health in young adults and vaccination may minimize COVID-19 induced alterations in vascular and autonomic function.

COVID-19 is an illness that the clinical and scientific community had to learn about and adapt to in real time since it was a novel disease. It was in this environment that patients themselves first coined the term “Long COVID”, a condition in which some individuals who had COVID-19 experienced persistent debilitating symptoms several weeks to months after the initial illness. Although these

symptoms were initially disregarded as inconsequential by many, later, this condition also now known as "post-acute sequalae of COVID-19 (PASC)" or "post COVID condition was officially given a clinical case definition by the World Health Organization (51) which helped clinicians to make the diagnosis, patients to receive appropriate treatment, and researchers to study it. It is estimated that depending on the variant, between 20 – 50% of individuals suffer from Long COVID. A higher number of cases are seen among females (52), but it occurs in all age groups and those with all levels of initial COVID-19 severity including a significant number of those who had only mild acute illness (53). PASC has been associated with over 50 symptoms and complications including cardiovascular and cerebrovascular effects (54). Therefore, in the next study, we aimed to investigate peripheral and cerebral vascular function and central arterial stiffness in middle-aged females with a diagnosis of PASC and age-matched controls without PASC, and to determine whether there is a relationship between the above measures and PASC symptom burden (55). Female patients with a physician diagnosis of PASC and those with no evidence on PASC were recruited. Measures of peripheral and cerebral vascular function and arterial stiffness were assessed as described previously. PASC symptoms and their severity were also recorded, and a total symptom burden was calculated as the sum of the severity score of each symptom. In addition, we assessed their physical function using a questionnaire and 6-min walk test and quality of life also using a questionnaire. Note that most of these patients did not require hospitalization for the acute COVID-19 illness and were studied at a median time of 15 months past their initial COVID-19 diagnosis. Our findings revealed that there was no impairment in peripheral or cerebral vascular function in patients with PASC. However, we did observe that patients with PASC had significantly higher peripheral blood pressure, central blood pressure, and central arterial stiffness compared to the control group suggesting that there could be long-term effects of PASC on cardiovascular health (55). In addition, we found that measures of physical functioning and quality of life were significantly correlated with the total symptom burden such that those with a greater symptom burden had worse physical function and worse quality of life.

At this point, to briefly summarize what has been presented thus far on COVID-19 and cardiovascular health, earlier variants of COVID-19 exert a persistent negative impact on peripheral vascular function even in young otherwise healthy adults. These effects on peripheral vascular function appear to be related to persistent symptoms, whereas effects on arterial stiffness, blood pressure, and cardiac autonomic function appear to be minimal and transient. In relation to long COVID or PASC, these patients may be at a higher risk for development of hypertension and other cardiovascular diseases.

Autonomic Function Testing Laboratory, University of Peradeniya

In the next section, I will divert your attention to what we do at the autonomic function testing laboratory at University of Peradeniya. The foundation to this work was initiated in 2017, after attending the workshop on physiological techniques at AIIMS, New Delhi. Together with Prof. Indu Nanayakkara, one of my MPhil supervisors at that time, we set out to establish the first autonomic function testing laboratory in the country, at the Department of Physiology, Faculty of Medicine, University of Peradeniya. Of course, this was no easy task, but with the support of the then Head of the Department Professor Sudheera Kalupahana, Dean, Professor MD Lamawansa, and research grants we obtained, within a short period we were able to equip the lab with state-of-the-art equipment and data analysis software that are essential for basic autonomic function testing. Work in the lab is carried out with the help of three well-trained technicians, Ms Kaushalya, Ms Ishanthi, and Ms Himali. To date, we have provided cardiac autonomic function testing for over 250 patients at the request of physicians. These tests assist in the diagnosis and management of autonomic dysfunction in a variety of patients.

In addition to providing clinical services, the lab is also utilized for undergraduate and postgraduate teaching and research. It is well known that there are ethnic and racial differences in cardiovascular function and disease risk (56,57). Therefore, one of

the first tasks that was undertaken after establishment of the lab was to develop normative data for cardiovascular autonomic function tests for the Sri Lankan population. For this, we perform the standard cardiovascular autonomic reflex tests mentioned previously in healthy adults over 18 years and calculate the indices of autonomic function from each test. While some preliminary data was presented at the 8th Biennial Conference of the South Asian Association of Physiologists, held at BMICH, Colombo in 2022, we continue to recruit participants to expand our database.

Another ongoing study in the lab aims to identify the impact of age and body position on the cardiovascular response to the Valsalva maneuver. This is important since, depending on patient comfort, and the facilities available in the lab, autonomic testing is performed in different positions including the supine, seated, or at a tilted position in different labs (58). However, it is known that body position has an effect on autonomic function and therefore, it is not possible to interpret the findings without knowing whether the body position exerts a clinically significant impact on the tested autonomic function. Therefore, a simple study was designed to investigate the impact of body position on the cardiovascular response to Valsalva maneuver. For this we are recruiting healthy adults above 18 years and performing the Valsalva maneuver in 3 positions, namely supine, 20 degree and 30-degree tilt. The preliminary data suggests that the tilt position indeed does impact the interpretation of the Valsalva response to a clinically significant degree in young adults (59). We are continuing this study to investigate older age groups and plan to extend the study to patient populations.

In addition to these studies that focus on fine tuning the output from the lab, several studies investigating autonomic and vascular function in at-risk populations are also underway in the lab. This includes the assessment of cardiac autonomic function in hypertensive patients and a study investigating cardiac autonomic function and blood pressure variability in individuals with a family history of hypertension. Collectively, the work that is being carried out at the autonomic function testing lab at the Department of Physiology is paramount to improving the quality of life of patients and better understanding the potential risk factors for cardiovascular disease in Sri Lankans. My ultimate goal is to expand the autonomic testing lab to the first autonomic and vascular function assessment lab in Sri Lanka, which I hope to achieve soon with funding that I will hopefully receive in the near future for a recently submitted multi-disciplinary research proposal.

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