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Letter to Editor

# Nightingale Visits the 21st Century

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## Introduction: A return, not a resurrection

Florence Nightingale (1820–1910) is formally regarded as the originator of contemporary nursing and a forerunner of evidence-based health care [1]. During the Crimean War, she provided revolutionary advances in the standards of sanitation, the process of hospital reform, and systematic thought in healthcare in a way that made her one of the earliest practitioners of the principle of combining scientific observation and practice [2]. However, as we enter the 21st century with perplexing and rapidly advancing technology and science, we continue to adopt or abandon Nightingale's foundational principles in a way that is not applied comprehensively and in various systems of healthcare.

Recent global health challenges, especially the COVID-19 pandemic, have generated systemic susceptibilities within public health infrastructure and expounded the limits of nursing capacity and health equity, a point Nightingale made and emphasized over 150 years ago [3]. Nightingale asserted that health systems should be built on treatment, education, sanitation, and community-based care [2]. However, these principles are often overlooked or overshadowed by an increasing dependence on rapid technology.

An imagined return of Nightingale to our century would therefore provoke not only admiration for the innovations in biomedicine and digital health but also disappointment at how the essential lessons of environmental health, holistic nursing, and social justice in care have been sidelined. Simply, the challenges of today's global health landscape, including pandemics, inequities in access to care, and the climate crisis, are not entirely new; they echo problems Nightingale identified long ago. The unsettling reality is that, despite our progress, we missed a lot of what she already knew.

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**Sanitation and environmental health**

One of Nightingale's most groundbreaking contributions to healthcare was her understanding of the relationship between the environment and patient outcomes [1]. Clean air, safe and effective ventilation, cleanliness, and space formed the basis of her model of care [2]. However, long before the COVID-19 pandemic brought renewed attention to the mode of airborne transmission, many modern hospitals had evolved into increasingly conditioned, crowded, and poorly ventilated environments, focusing on the efficiencies of technology rather than the environment in which care is provided.

Since Nightingale's era, many sanitary conditions have improved. However, many contemporary environmental health challenges have emerged or intensified. Currently, air pollution, food safety concerns, radiation exposure, and climate change-related health impacts are major public health threats. The public health infrastructure worldwide has deteriorated, particularly in many contexts, owing to budgetary deficits and austerity [4]. Nightingale would have been horrified to see that basic public sanitation and hygiene systems, preventive efforts, and education about the importance of cleanliness, major successes of 19th century reform, are now often taken for granted and neglected.

**Data, statistics, and the superficial use of evidence**

Nightingale's use of statistics was both inventive and transformative, making complex data understandable for policymakers in a way that influenced healthcare reform [5]. Nevertheless, in the 21st century, the sheer volume of health data may not have led to much more actionable, preventive, or compassionate care. Rather, research and data are commercialized, institutionalized in proprietary packages, or used as weapons of mass budget reduction [6].

Additionally, Nightingale's belief in the integrity of person-centered observational data (the person, their story, their context) has in some settings been overshadowed by standardized protocols, metrics, and algorithm-driven systems. Currently, we have tools that Nightingale would have marveled at, but we often lack her focus on using evidence to alleviate suffering rather than controlling budgets or bureaucracy.

**Public health: A forgotten battlefield**

Nightingale stated that hospitals should be understood as "temporary homes for the sick" and not as permanent resting places for care, as if it were a hospital duty. The ultimate battle for health is within communities based on their foundations: sanitation, nutrition, housing, and education [2]. Nightingale's vision incorporated prevention and clearly asserted that the determinants of health actually lie outside of hospital walls.

In the 21st century, we have largely neglected this ancient wisdom. It may be due to a contemporary Western health system that is now excessively hospital-based, curative, and reliant on technology. The COVID-19 pandemic highlighted the precariousness of this model, and for a moment, the world was reminded of the importance of public health measures, including access to clean water, proper hygiene habits,

accessible community education, and fair and ethical resources to safeguard populations.

Nonetheless, many countries still have public health systems that are consistently under-resourced and politically marginalized, valued beneath biomedical and hospital-based care [7]. Often, investment in preventive programming by communities is minimal; government and political priorities reflect short-term economic as opposed to longer-term population health goals. This inability to invest in preventive programming not only diminishes preparedness for crises but also maintains cycles of inequity, where the burden of disease is shared disproportionately in disadvantaged communities. The neglect of preventive, community-based care mimics the loss of the core insight of Nightingale's most systemic insight, that health is determined much more by the social and environmental conditions in which people live, work, and grow than by what may happen in a hospital ward.

**Nursing as an intellectual and moral discipline**

Nightingale envisioned nurses not as subordinates to physicians but as educated, autonomous professionals trained in both science and ethics [1,2]. In many parts of the modern world, nursing has struggled to assert this identity. The profession has been undervalued, often feminized and bureaucratized, rather than empowered.

Irrespective of the huge demand for nurses, there is a current trend to decrease the nursing workforce. And also, the field of nursing is under chronic underinvestment worldwide, considering its education, mental health, and leadership development.

**Digital health**

If Nightingale observes electronic health records, artificial intelligence, and telehealth, she would probably not be surprised. But her first question would be “Are those supporting the patient or the system?” She would not question or criticize the technology, but she would probably be concerned about the extent to which these innovations affect ethical reasoning and decision-making in patient care. In the 21$^{st}$ century, we value innovations and technologies, we value large data sets, and we make decisions or train Artificial Intelligence models. However, there is a concern about whether we truly use this data to create a risk stratification or to identify vulnerable individuals, or we are merely creating efficiencies in the system. At that point, there is a concern, are those systems more reliable? Are we creating hospitals as a place to heal the sick or just collecting some machines to improve efficiencies?

**Conclusion**

The legend of Florence Nightingale is not just a story of the past. It is a great reflection of today's healthcare. She uplifted healthcare services during the 19$^{th}$ century by developing fundamental principles of sanitation, observation, compassion, and social equity. All her practices and innovations were based on rigorous scientific methods. However, in the 21$^{st}$ century, implications and rapid advancement may neglect her compassionate caring principles. We are now over quality. If Nightingale visited us today, she

may find inspiration, but at the same time, indictment as well. She would recognize many of the tools we currently use, but she would question the motives behind those tools themselves. Florence Nightingale would applaud what we can do with the massive data we are now able to obtain from our patients, and she would question what we are doing with that data. Nightingale would not suggest we reinvent medicine, healthcare, or nursing; instead, she would ask us to remember the fundamental principles of compassionate, ethical, and patient-centered care. Nightingale's vision still stands today in rich relevance, implying we need not venerate her name, but implement her ideas. There is a huge demand for nurses, and we are talking about decreasing the nursing workforce as well. Yet the field of nursing is under chronic underinvestment worldwide, considering its education, mental health, and leadership development.

**Declaration of conflicting interest**

The authors declare that they have no conflict of interest.

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Original Research

# Excessive Screen Time and Its Association with Psychological Health among Adolescents in Galle Educational Division, Southern, Sri Lanka

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## Abstract

The rise in excessive screen time and addictive behaviour among adolescents is a growing concern that significantly affect their psychological well-being. Identifying the extent of excessive screen time and its association with psychological problems is crucial for developing effective policies. A descriptive cross-sectional study was conducted among 420 schoolchildren aged 12-16 from selected schools within the Galle Educational Division, using a stratified multistage cluster sampling to assess the prevalence of excessive screen time and its association to the psychological health among adolescents in the Educational Division of Southern, Sri Lanka. A pretested, self-administered questionnaire was used to assess the addictive behaviour. Depression Anxiety Stress Scale - Youth 21 (DASS Y-21) was used to assess the severity of depression, anxiety, and stress symptoms. Data were analysed using SPSS 25.0, and the Chi-square test, and binary logistic regression were performed. Of the sample, 39.6% used screen devices for more than the recommended 2 hours, except for educational purposes. Smartphones (20.6%) and television (25.5%) were the most frequently used devices. The majority (45.1%) of the participants indicated that they routinely check their device screens in the absence of any incoming notifications, while 38.9% of participants reported increased screen time, and 27.7% reported constantly feeling an urge to use them. Mother's occupation ($p$=0.05), parental screen devices usage ($p$=0.01), the availability of television ($p$=0.01), and the use of screen devices while eating ($p$=0.001) were associated with excessive screen time. Among the participants, 23.4% had symptoms of severe depression, 18% had severe anxiety, and 19.8% had symptoms of severe stress. Excessive screen usage is significantly associated with depression ($p$=0.028), anxiety ($p$=0.048), and stress ($p$=0.002) among adolescents. Excessive screen time, addictive behaviour and psychological problems are substantially prevalent among adolescents. Awareness, behavioural modification intervention, and balanced screen usage are crucial for promoting their well-being.

***Keywords***: *Adolescents, Addictive behaviour, Excessive screen usage, Psychological impact*

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## Introduction

Extensive screen use, including smartphones, computers, tablets, and televisions, is often operationally defines in literature as recreational screen time exceeding two hours per day, excluding educational activities [1]. This high exposure to screen time has a potential risk of developing problematic screen usage. Problematic screen usage is defined as a person's inability to control the use of screen devices despite their harmful consequences [2]. It is also referred to as technology addiction, digital addiction, describing obsessive, unhealthy screen use.

Over the past decades, there have been rapid behavioural trends among adolescents, including unprecedented changes in increasing screen use. The COVID-19 pandemic, the availability and accessibility of digital devices, and the excessive use of social media, online purchasing, online learning, and digital entertainment further accelerated this trend. Teens are more susceptible to excessive screen usage due to parental, peer, and social influences. Adolescents often suffer in social interactions, physical health, academic performance, and overall well-being because of this [3]. According to the existing research, the prevalence of problematic screen addiction usage among adolescents globally is estimated to be 26.9%, with smartphone use being the most associated device [4]. However, currently, there has been limited reported data on the prevalence of excessive screen time usage specifically among adolescents in Sri Lanka.

Several studies have been conducted in the South Asian region to study the level of screen addiction among adolescents. A cross-sectional survey done in India reported 10.69% of technology addiction, and it was indicated that almost one in ten adolescents had mobile addiction [5]. karnata [6]. Another study conducted in China found that 33.3% addicted to the internet and revealed that female gender, age, depression, and stress were all significantly correlated with Internet addiction [7].

Excessive screen usage and screen addictive behaviours cause many psychological consequences among adolescents. Depression is a significant public health concern and a common health problem. Adolescents are more prone to have psychological disorders like depression, anxiety, and stress. Generally, depression is linked with stress, and adolescents are more prone to use screen devices to reduce their stress. According to the previous studies [8,9,10], overall screen time has been linked to depression in adolescents, and a nonlinear dose-response relationship between depressive symptoms and overall screen time was discovered in adolescents who used digital media for more than two hours per day. Some research studies revealed that adolescents who were addicted to the internet had high rates of psychiatric comorbidity, particularly behavioural, anxiety, and mood disorders [11]. A cross-sectional study conducted among adolescents in South Karnataka found that 3.9% were addicted to screens. Furthermore, males showed a higher prevalence of addiction than females, as they have been addicted to online gaming. Also, the same study reported that adolescents with probable depression were five times more likely

to be addicted to screens than those who do not have depression [2].

In Sri Lanka, there has been a significant rise in the number of adolescents seeking help from child and adolescent mental health services for issues related to excessive screen use, and many complaints from teachers and parents about this issue [12]. The island-wide programme has been launched and activated through the Medical Officer of Health office (MOH), in collaboration with the educational ministry, to support vulnerable adolescents and prevent addiction through educational and awareness programmes. Recently, the government of Sri Lanka issued policy recommendations to discourage children from using social media. Despite all these implications, the problem remained unchanged, with evidence of victim children present before psychological help, and showing poor performance in their education.

There are a few studies conducted in the Sri Lankan context to assess the gravity of excessive screen usage and its association with the psychological health of adolescents. Policymakers and health professionals require adequate evidence-based information to implement sustainable programmes to address this at the population level. Therefore, this study was conducted to address this gap, and it aimed to assess the prevalence of excessive screen usage and its association in psychological health of adolescents in the Galle educational division, Sri Lanka.

**Methods**

A descriptive cross-sectional study was conducted involving 420 secondary-level school children in the Galle educational division. Both male and female adolescents at the age of 12 - 16 years were interviewed. Six randomly selected schools in the Galle Educational Division, including one girls' school, one boys' school, and four mixed schools, were used for the data collection. The sample size was calculated based on an estimated 68% prevalence of psychological problems among adolescents, drawing on previous literature. A 420-sample was considered, adding a 10% non-response rate to the calculated sample size.

**Selection criteria**

Both male and female school children from selected schools in the Galle educational division, aged 12 - 16 years (grades 7 - 11), who can understand English or Sinhala, were considered for inclusion. Students who are absent during the data recruitment period (long-term absence due to any medical issues), and those who are getting treatment for depression, anxiety, or have a significant disease condition, were excluded from the study.

**Study instruments**

A multi-sectioned questionnaire was used as the study instrument. This consisted of four parts, including demographic data, questions to assess excessive screen usage, screen addictive behaviour, influencing factors for excessive screen usage, and psychological problems experienced by the adolescents. Usage of any screen device for more than the recommended 2

hours for recreational activities, except for educational purposes, was used to assess excessive screen usage among the adolescents. A self-developed 5-point Likert scale comprising 13 questions was used to identify addictive behaviour after reliability and pretesting the questionnaire (Cronbach's Alpha reliability was 0.874). The severity of symptoms of depression, anxiety, and stress were assessed using DASS-Y 21, which is a validated screening instrument to assess adolescents, and which consists of 21 items [13]. The tool was cross-culturally adopted for use in the Sri Lankan context, with pretesting, and it demonstrated good internal consistency (Cronbach's Alpha reliability was 0.904). This scale gives the level of depression, stress, and anxiety as normal, mild, moderate, severe, and extremely severe.

**Data collection procedure**

Data collection was done after obtaining institutional permission for the study. Six out of thirty-one schools were purposefully selected as planned, and predetermined inclusion and exclusion criteria were considered when recruiting the samples. Participants for the study were selected randomly through a multi-stage cluster sampling. Information was given to both participants and parents, and written consent was obtained from parents and children. They were informed of the dates and times for the data collection. Data collection continued without disturbing their academic work, and students were given only 20 minutes to complete the questionnaire.

**Data analysis**

Data were analysed using SPSS version 25.0. Missing values of the categorical data were replaced with the mode. The prevalence of excessive screen usage and addictive behaviour were presented as frequencies and percentages. The association between influencing factors for excessive screen usage was tested using the Chi-square test. Binary logistic regression was performed to determine the likelihood of psychological consequences for excessive screen usage, and adjusted odds ratios with 95% confidence intervals (CIs) were obtained. A *p*-value of 0.05 was considered statistically significant for all analyses.

**Results**

A total of 420 participants were surveyed, with a response rate of 99.7% (n=419). Among them, the majority were Sinhalese (81.1%), and 56.6% were males. Age distribution among the participants was: 12 years old (17.7%), 13 years old (18.1%), 14 years old (20.5%), 15 years old (22%), and 16 years old (21.7%). Adolescents aged 15 years (25.3%) and 16 years (25.3%) were predisposed to excessive screen usage compared to others.

**Excessive screen usage**

The prevalence of excessive screen usage was 39.6%. When considering the screen devices, as shown in Table 1, 3.6% used computers, 4.8% used laptops/tablets, 20.6% used smartphones, and 25.5% used television (>2hrs).

**Table 1** Descriptive statistics for usage of each screen device for more than 2 hours

| Variables | n | % |
|---|---|---|
| Use of computers | 15 | 3.6% |
| Use of laptops/tablets | 20 | 4.8% |
| Use of smartphone | 86 | 20.6% |
| Use of television | 107 | 25.5% |

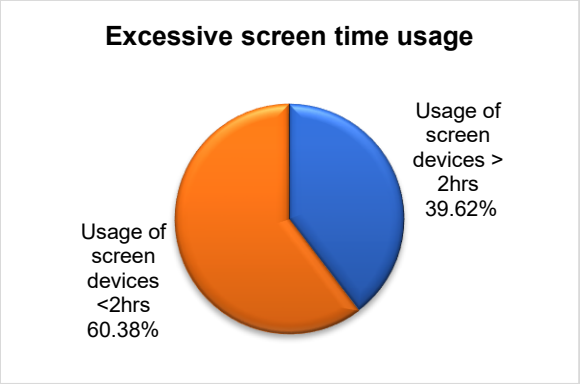



**Figure 1** Distribution of adolescents by daily recreational screen time (≤2 hours and >2 hours)

### Screen addictive behaviour

As illustrated in Table 2, 45.1% checked their device screens without an incoming notification, while 38.9% reported increased screen time. 32.2% used the devices without a specific task, and 27.7% reported feeling a constant urge to use them. Additionally, 21% reported that they need to turn on the television even though they have not used it.

**Table 2** Screen addictive behaviour of the participants (n=419)

| Statements | n (%) |
|---|---|
| I spend too much time with any of the screens | 103 (24.6) |
| I often use screen devices soon after waking up (while staying in bed) | 43 (10.2) |
| I often use screen devices before going to sleep (while staying in bed) | 81 (19.4) |
| I cannot stop using these devices. | 79 (18.9) |
| The time I spend has gradually increased. | 163 (38.9) |
| I used to use these devices even though I did not have a specific task with them | 135 (32.2) |
| Even if I do not get any message, I check the screens (phone, tablet, etc.). | 189 (45.1) |
| I constantly feel an urge to use some devices | 116 (27.7) |
| Even if I do not watch TV, I still want to switch it on. | 88 (21.0) |
| Even if I try to control or limit screen use, I cannot do that | 58 (13.8) |
| I feel like I do not have enough time for academic work due to the constant use of those devices | 79 (18.9) |
| I often get angry when someone suggests me to reduce/stop using screen devices. | 80 (19.1) |

### Influencing factors for excessive screen usage

Table 3 shows that the factors related to the mother's occupation ($p$=0.05), television availability ($p$=0.01), screen device use while eating ($p$=0.001), and parental screen device use ($p$=0.01) were significantly associated with excessive screen usage.

### Association with psychological status

Table 4 demonstrated that depression, anxiety, and stress were associated with excessive screen usage among the adolescents ($p$=0.028,0.048,0.002, respectively).

**Predictors of excessive screen time usage and psychological health**

According to table 5, adolescents with high screen time were significantly more likely to experience depressive symptoms compared to those with low screen time (OR=1.240, *p*=0.003, 95% C.I.=1.074-1.431)). Similarly, high screen time was associated with increased odds of anxiety (OR=1.241, *p*=0.005, 95% C.I.=1.069-1.441) and stress (OR=1.383, *p*=0.000, 95% C.I.=1.174-1.628).

**Table 3** Association of the influencing factors with excessive screen usage (n=419)

| Variables | | Excessive screen usage | | *p* value |
|---|---|---|---|---|
| | | No n (%) | Yes n (%) | |
| Ethnicity | Sinhalese | 203 (59.7) | 137 (40.3) | 0.557 |
| | Muslims | 50 (63.3) | 29 (36.7) | |
| Gender | Male | 144 (60.8) | 93 (39.2) | 0.857 |
| | Female | 109 (59.9) | 73 (40.1) | |
| No of family members | 3-5 | 67 (63.3) | 97 (36.7) | 0.276 |
| | 6-8 | 82 (55.8) | 65 (44.2) | |
| | >9 | 4 (50) | 4 (50) | |
| Occupation of the father | Government job | 57 (66.3) | 29 (33.7) | 0.208 |
| | Private job | 80 (57.1) | 60 (42.9) | |
| | Own job | 107 (61.1) | 68 (38.9) | |
| | Abroad | 9 (50) | 9 (50) | |
| Occupation of the Mother | No job | 178 (57.1%) | 134(42.9%) | **0.050** |
| | Government job | 46 (76.7%) | 14 (23.3%) | |
| | Private job | 20 (66.7%) | 10 (33.3%) | |
| | Own job | 7 (58.3%) | 5 (41.7%) | |
| | Abroad | 2 (40%) | 3 (60%) | |
| Educational level of the mother | Primary education | 6 (46.1) | 7 (53.8) | 0.301 |
| | Secondary education | 121 (57.9) | 88 (42.1) | |
| | Tertiary education | 126 (64) | 71 (36) | |
| Educational level of father | Primary education | 7 (38.8) | 11 (61.1) | 0.196 |
| | Secondary education | 135 (60) | 90 (40) | |
| | Tertiary education | 11 (63.1) | 65 (36.9) | |
| Family Income (LKR) | Poor (<50,000) | 70 (65.4) | 37 (34.6) | 0.217 |
| | Satisfactory (>50,000) | 183 (58.7) | 129 (41.3) | |
| Place of living | Home | 243 (60.6) | 158 (39.4) | 0.219 |
| | Other | 10 (55.6%) | 8 (44.4%) | |
| Participation in online learning | Yes | 63 (60.6) | 41 (39.4) | 0.963 |
| | No | 190 (60.3) | 125 (39.7) | |
| Use of television | Yes | 194 (55.7%) | 154 (44.3%) | **0.01** |
| | No | 59 (83.1%) | 12 (16.9%) | |
| Use of computers | Yes | 80 (55.9%) | 63 (44.1%) | 0.181 |
| | No | 173 (62.7%) | 103 (37.3%) | |
| Use of laptops | Yes | 83 (54.6%) | 69 (45.4%) | 0.068 |
| | No | 170 (63.7%) | 97 (36.3%) | |
| Use of smartphones | Yes | 233 (59.3%) | 160 (40.7%) | 0.075 |
| | No | 20 (76.9%) | 6 (23.1%) | |

| | | | | |
|---|---|---|---|---|
| Use of tablets | Yes | 29 (52.7%) | 26 (47.3%) | 0.213 |
| | No | 224 (61.5%) | 140 (38.5%) | |
| Parental usage of devices | Yes | 147 (53.1) | 130 (46.9) | **0.01** |
| | No | 106 (74.6) | 36 (25.4) | |
| Use of screen devices when eating | Yes | 86 (50.0) | 86 (50.0) | **0.001** |
| | No | 164 (68.0) | 77 (32.0) | |
| | Sometimes | 3 (50.0) | 3 (50.0) | |

*Note: p is from Pearson's Chi-square test that tested the association between influencing factors with excessive screen usage*

**Table 4** Association between excessive screen usage with depression, anxiety, and stress

| Psychological status | | Excessive screen usage | | *p* value |
|---|---|---|---|---|
| | | **Yes** n (%) | **No** n (%) | |
| Depression | Normal | 68.1 (113) | 79.8 (202) | 0.028 |
| | Moderate | 8.4 (14) | 7.5 (19) | |
| | Severe | 12 (20) | 7.1 (18) | |
| | Extremely severe | 11.4 (19) | 5.5 (14) | |
| Anxiety | Normal | 52.4 (87) | 67.0 (170) | 0.048 |
| | Mild | 11.4 (19) | 8.3 (21) | |
| | Moderate | 18.1 (30) | 13.4 (34) | |
| | Severe | 7.8 (13) | 4.7 (12) | |
| | Extremely severe | 10.2 (17) | 6.3 (16) | |
| Stress | Normal | 50.0 (83) | 66.8 (169) | 0.002 |
| | Mild | 14.5 (24) | 13.0 (33) | |
| | Moderate | 15.7 (26) | 10.3 (26) | |
| | Severe | 12.0 (20) | 7.9 (20) | |
| | Extremely severe | 7.8 (13) | 2.0 (5) | |

**Table 5** Logistic regression model of predictors (n=419)

| **Predictors** | **OR (95% CI)** | ***p* Value** |
|---|---|---|
| Depression | 0.215 (1.074-1.431) | 0.003 |
| Anxiety | 0.216 (1.069-1.441) | 0.005 |
| Stress | 0.324 (1.174-1.628) | 0.000 |
| $X^2$ | 8.710 | |
| H-L goodness of fit 0.210 | 0.900 | |
| -2 Log | 553.950 | |

*[2]Note: H-L GF-Hosmer – Lemeshow goodness of fit statistics, $X^2$-Chi-square, -2log=-2log likelihood <0.05*

## Discussion

This study was conducted to assess the prevalence of excessive screen usage, addictive behaviour, and its associated psychological problems among adolescents in Galle Educational Division, Southern Sri Lanka. The findings of the study revealed that higher screen usage among the 15 - 16-year-old category, and parental screen usage and psychological status on depression, anxiety, and stress are associated with excessive screen usage among adolescents.

This study provided a prevalence of 39.6% excessive screen usage, which predominantly affects the 15-16-year age category. Similar findings were reported in several studies, which coincide with the present study reported 39.6%, and 24.4% prevalence of high screen usage in the same age category [3,14]. This may happen naturally due to involvement with their peers' social contacts through screen devices. Biological, socio-environmental, emotional, and psychological factors largely influence decision-making in this age category. As a recent Sri Lankan study revealed, a considerable number of (41.4%) community of overweight and obese teenagers (aged 10 to 14) reported having a higher level of dependence on technology, due to physical appearance, which proves that the teenagers' psychological influences on addictive behaviour [15]. Additionally, teenagers prefer mobile phones as multi-tasking devices, and they are readily accessible across different socio-economic groups. They are also highly attracted to technological devices due to their innovative nature. However, in contrast to the current study, some studies reported prevalence rates of 3.9% to 13.1% across their studies [2,5,15,16] which found that results variation due to social background and different sample sizes and survey times utilized by those studies followed different methodologies. In general, it was revealed that the 15 to 16-year-old age category needs more attention and support for controlling their addictive behaviour, and further studies are recommended within the different teenage categories, covering different socio-economic and geographical strata, to propose an actual prevalence of screen addiction among this population group.

Besides, parental screen usage is influential for adolescents screening addictive behaviour as they purposefully mimic their parents. This study found that parental screen usage is significantly associated with children's screen addiction. Previous studies consistently support this finding [16]. The current study findings are in line with an Asian study conducted in China [17], which emphasized that parents are more focused on their screens and neglecting their children. Teenagers who have modelling behaviour mostly learn by observing their parents. If parents are always on their phones, children learn it as normal and desirable. Parents should be good role models to their children by limiting their own screen time and staying away from screens during parenting time to expect good results.

Socio-economic status is highly influential on addictive behaviour among teenagers [18]. However, the current study findings are not supported with the above previous literature findings. Recent Indian studies have revealed that the majority of the lower or lower-middle class, who were from nuclear households, were mainly affected by screen addiction[2,19]. Moreover, male students who live in rural regions and use smartphones were more prone to screen addiction [20]. The ages of 15-16 were the most significant age groups, and there is a piece of evidence that this risk is increased by indicators of lower socioeconomic position, such as lower mother education and single-parent households [3,21]. Parents with low socioeconomic status have lower digital literacy, leading to less supervision of the children's screen use.

Additionally, children with limited social interactions have fewer alternatives and are more vulnerable to addiction. Social pressure, bullying, and negative social comparison make them feel more cornered in the society, leading to excessive screen use. Further studies are required to determine predisposing factors for screen addiction among teenagers in different socio-economic groups.

This study assessed psychological status, including depression, anxiety, and stress, among the study's population, and found that a considerable proportion of the adolescents were experiencing moderate to severe depression, anxiety, and stress. Globally, a substantial rate of adolescents is suffering from the above psychological problems [22], often linked with the socio-economic disparities, unrealistic future goals, self-esteem issues, and maladaptive behaviours [23]. The current study findings show that there is a strong and significant association between the above-mentioned psychological status and excessive screen usage, similar to the previous studies. Several studies have shown that a moderately favourable connection exists between screen time and the severity of depression, anxiety, and stress symptoms [24,25]. Besides, anxiety was significantly higher in students who used the internet for texting, entertainment, and news [7]. Some of the studies found that Boys who played video games for 6 or more hours had higher anxiety symptoms ($p$<.05), with a large effect size (OR=5.12, 95% CI: 1.56-17.44). Social movement restrictions, such as those during COVID-19, also significantly affect psychological status by limiting face-to-face interactions [26]. Overall, screen overuse reduces in-person social interactions and leads to social isolation, which ultimately contributes to psychological imbalance.

As shown in the current study and the previous studies, a substantial proportion of adolescents are reported to have depression, anxiety, and stress [7] Adolescents experiencing fluctuating moods, and some teenagers are unable to manage them, leading to stress, anxiety, and ultimately to depression. These psychological situations forced them to adopt alternative solutions. Nowadays, teenagers choose screens as an alternative, and they are prone to excessive screen use. According to the current study, anxiety predicts a 1.1 times likelihood of getting addicted to the screen in adolescents. Finally, adolescents with psychological disturbances are getting addicted to screens, and those who are addicted are also more prone to developing psychological issues. However, the current study is a cross-sectional survey, which is limited in its ability to predict causal factors influencing screen addiction. It is suggested that conducting correlational studies to determine the causative factors will disclose the real picture of screen addiction among adolescents in Sri Lanka.

**Limitations**

A national-level sample was required to generalize the findings, as this study was limited to the specific geographic location**.**

**Conclusion**

Excessive screen usage among adolescents is a significant issue. The parental background, availability of screen devices, and unhealthy eating behaviours were found to be the vital

determinants of excessive screen usage. Also, it was evident that there were some psychological consequences associated with excessive screen usage. On the other hand, the psychological status of the adolescent might lead them to spend more time in front of screens. Therefore, it is strongly recommended to provide ongoing psychological support to adolescents, including mental health screening, counselling services, and psychological treatment. It also recommended conducting awareness programs on excessive screen usage and its consequences for both parents and adolescents. The study's findings are highly recommended for use by health and education policymakers and program developers in developing preventive and treatment measures.

## List of abbreviations

Confidence Interval-CI
Depression Anxiety Stress Scale-DASS Y-21
Medical Health Officer – MOH
Statistical package for social sciences- SPSS

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## Ethical consideration

Ethical approval for the study was obtained from the Ethical Review Committee of the Faculty of Allied Health Sciences, University of Ruhuna, Sri Lanka (Ref. 155.11.2022). Institutional permission for the study was obtained from the Provincial Director of the Educational Department, the Zonal Director, the Divisional Director, and the principals of the schools. All participants and their parents were provided with an information sheet about the study and obtained written consent for participation.

## Conflict of interest

Authors declare that there is no conflict of interest.

## Author contributions

All authors contributed to the study conception and design. Material preparation, data collection, and data analysis were performed by PKTPP. The first draft of the manuscript was written by PKTPP, and BIW. All authors read and approved the final manuscript.

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Original Research

# Prevalence of Anemia and Its Association with Body Mass Index Status among Allied Health Sciences Students at a Leading University in Sri Lanka: A Cross-sectional Study

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## Abstract

Anemia and obesity are major global health issues associated with substantial mortality in developing countries. Although the student population was reported to be more vulnerable to anemia and Body Mass Index (BMI) outside the normal range, there is a lack of contemporary data in Sri Lanka. Thus, the present study aimed to investigate the prevalence of anemia in relation to BMI status among students in the Allied Health Sciences (AHS) at a leading university in Sri Lanka. After obtaining informed consent, blood samples were collected, and hemoglobin concentration was estimated. Weight and height were measured, and BMI was calculated. Additionally, a self-administered questionnaire was utilized to gather data on factors associated with obesity and anemia. Results revealed a prevalence of 22.9% for anemia and 50.1% for underweight or overweight/obesity, with a female predominance. Furthermore, anemic individuals had significantly higher BMI levels than healthy students ($p = 0.028$), although no significant differences were found between BMI categories and anemia status. The risk analysis revealed that students with BMI outside the normal range have a higher chance of being anemic than those with normal BMI. Irregular exercise and less consumption of green leafy vegetables were associated with anemia ($p < 0.05$), while a family history of anemia and obesity showed a significant association ($p < 0.05$) with obesity. In conclusion, the prevalence of anemia among AHS undergraduates with higher BMI levels is relatively high; however, further studies are recommended to find the association between obesity and anemia.

**Keywords:** *Anemia*, *Body Mass Index*, *Hemoglobin*, *Students*

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## Introduction

Anemia, defined by hemoglobin (Hb) concentrations below 12.0 g/dL in adult females and 13.0 g/dL in males by the World Health Organization (WHO), is one of the leading healthcare problems in developing countries [1]. Approximately 40% of children, 37% of antenatal women, and 30% of women aged between 15 and 49 years were affected by anemia in these nations [2]. In Sri Lanka, the national prevalence of anemia was reported as 29% by the WHO; however, the only national survey done in 2006 revealed that 20.7% and 13.3% of Sri Lankan females had mild and moderate to severe anemia, respectively [3, 4]. It was widely reported that factors, such as low dietary iron absorption, helminth infections, multiple pregnancies, postpartum hemorrhage, practicing veganism due to religious beliefs, living in urban areas, gastric ulcers, menorrhagia, and low socioeconomic status, were associated with anemia [5].

Obesity, by affecting approximately one in eight individuals in the world, has become another significant public health issue [6]. Obesity, defined by the WHO as a BMI of more than 30 kg/m², causes severe adverse health effects and reduced life expectancy due to excess body fat accumulation [7]. Furthermore, the increasing prevalence of obesity among adolescents was reported to contribute to the emergence of multiple obesity-related comorbidities and economic burdens in developing countries [8, 9]. Studies have shown that obese adults are at an increased risk of developing anemia due to obesity-induced chronic inflammation, insufficient dietary iron intake, and inflammation of fat cells [10, 11]. Nonetheless, some research suggests that anemia is less prevalent among obese teenagers, highlighting the ongoing debate regarding the correlation between BMI category and anemia across different demographic groups [12, 13, 14].

University students are vulnerable to being overweight or obese due to irregular and unbalanced dietary habits, as well as excessive consumption of fast foods [15]. Additionally, several studies have shown that anemia is more prevalent among students, who are also at a higher risk of developing serious health complications such as diabetes, heart disease, high blood pressure, high cholesterol, and cancer [16, 17, 18, 19, 20]. As a result, investigating the obesity related prevalence of anemia and its associated factors among university students is necessary for improving their overall health and academic performance. However, there is a lack of contemporary data on the prevalence of obesity and anemia among undergraduates at Sri Lankan universities. Therefore, the present study aimed to assess the prevalence of anemia in relation to BMI status among AHS students at a leading university in Sri Lanka. The findings of this study could contribute to improving the health status of students and enhancing their academic performance in the future.

## Methods

### Study design and study population

This was a single-center cross-sectional study conducted among 375 undergraduates aged between 20 and 29 years from the Faculty of AHS, University of Jaffna (UoJ), Sri Lanka. Participants were informed of the voluntary nature of the study, and written informed consent was obtained prior to data collection.

**Sample size and sampling technique**

The sample size was calculated using the Daniel formula, with a prevalence (p) of 0.326 obtained from the literature [21]. The calculated sample size was 375, assuming a 0.05 absolute error and a 10% non-response rate. At the time of sample collection, 651 AHS students were enrolled at the UoJ. Therefore, 375 participants were randomly selected using a random number generator and invited to participate in the study.

**Data collection and BMI calculations**

All male and female undergraduate students of the Faculty of AHS, UoJ, were considered for the study. Pregnant and lactating students and those with a history of hematological disorders were excluded. From the eligible students, 375 were randomly selected and invited via email to attend the laboratory in the Department of Medical Laboratory Sciences. A self-administered questionnaire, containing information on sociodemographic and lifestyle factors, was provided to the students after explaining their right to withdraw and obtaining written consent. Upon completion of the questionnaire, anthropometric measurements of height and weight were taken, and BMI was calculated. Body weight was measured without footwear to the nearest 0.05 kg on a standard weighing scale, while height was measured with a stadiometer to the nearest 0.5 cm, with the subject standing upright. BMI was calculated using the formula: BMI = Weight (kg) / Height ($m^2$).

**Blood sample collection and Hb estimation**

2 mL of venous blood samples were collected from participants by well-trained nursing staff in a comfortable, undisturbed, and clean environment, using EDTA bottles. Collected samples were transported without delay to the laboratory at the Department of Medical Laboratory Sciences, and Hb concentration was measured by the cyanmethemoglobin method [22]. In brief, 20 µL of EDTA blood was added to 4 mL of Hb reagent, thoroughly mixed using a vortex, and kept at room temperature for 5 minutes. The absorbance was measured at 540 nm against a Hb reagent blank, and the Hb concentration was calculated using a standard curve.

**Determination of anemia and BMI status**

The prevalence of anemia was estimated according to the WHO criteria, where anemia was defined as Hb levels of <12.0 g/dL in females and <13.0 g/dL in males [1]. Conversely, the incidence of overweight/obesity was established based on the Asia-Pacific classification, where BMI was categorized as underweight (<18.5 kg/$m^2$), normal weight (18.5–22.9 kg/$m^2$), overweight (23.0–24.9 kg/$m^2$), and obese (≥25.0 kg/$m^2$) [23].

**Statistical analysis**

Statistical analysis was accomplished using the Statistical Package for the Social Sciences (SPSS) version 18.0. The prevalence of anemia, overweight/obesity, and their associated factors were determined by analysing the data obtained from laboratory results and questionnaires. Associations between sociodemographic and other related factors with anemia and obesity were analyzed using the Chi-square test or Fisher's exact test. Mean differences in Hb and BMI levels were assessed using the independent t-test or one-way ANOVA. Risk analysis was performed, and interpretations were made based

on odds ratios. A *p*-value of <0.05 was considered statistically significant.

## Results

### Baseline characteristics of study participants

In the present study, 341 students participated with a response rate of 90.9%. Most of the participants (63.9%) were female, with a male-to-female ratio of 1:1.77. Furthermore, the higher percentage of participants were Sinhala (78.5%) and Buddhist (76.8%), compared to students of other ethnic and religious backgrounds. The sociodemographic characteristics of the participants are shown in Table 1.

**Table 1** Sociodemographic characteristics of participants (n=341). (Number of subjects and percentages)

| Socio-demographic characteristics | Number of subjects (n) | Percentage of subjects (%) |
|---|---|---|
| Age | | |
| 20-24 years | 151 | 44.3 |
| 25-29 years | 190 | 55.7 |
| Gender | | |
| Male | 123 | 36.1 |
| Female | 218 | 63.9 |
| Marital status | | |
| Married | 3 | 0.9 |
| Unmarried | 338 | 99.1 |
| Ethnicity | | |
| Sinhala | 268 | 78.6 |
| Tamil | 53 | 15.5 |
| Muslim | 20 | 5.9 |
| Religion | | |
| Buddhist | 262 | 76.8 |
| Hindu | 45 | 13.2 |
| Islam | 20 | 5.9 |
| Christian | 14 | 4.1 |
| Residence | | |
| External boarding houses | 143 | 41.9 |
| University hostel | 198 | 58.1 |
| Family income | | |
| ≤ 50,000 LKR | 233 | 68.3 |
| > 50,000 LKR | 108 | 31.7 |

*LKR, Sri Lankan Rupees*

### Prevalence of anemia associated with different categories of BMI

Overall, the mean Hb concentration of the participants was 138.23 ± 22.07 g/L, and the BMI was 22.18 ± 3.54 kg/m$^2$, which were generally under the normal levels. However, 22.9% of the students were found to be anemic, with the majority (12.3%) having mild anemia (Table 2). Furthermore, BMI analysis revealed that 50.1% of the participants had abnormal BMI, among whom 19.9%, 17.3%, and 12.9% were obese, overweight, and underweight, respectively (Table 2). Notably, the results found that anemia and abnormal BMI were more prevalent in female students, accounting for 35.3% and 50% of the total female population. The comparative analysis between different categories of BMI and Hb indicated that anemic individuals had significantly higher BMI levels compared to non-anemic participants ($p$ = 0.028) (Figure 1c), albeit there was no significant association between BMI categories and anemia status ($p$ = 0.675). Interestingly, risk analysis revealed that students with abnormal BMI had a higher chance of developing anemia (OR = 1.149, 95% CI: 0.778–1.698) compared to those with normal BMI. Notably, a higher prevalence of anemia (6.59%) and a greater risk ratio of 1.309 (95% CI: 0.814–2.104) were observed among obese students (Figures 1a and 1b).

### Factors associated with anemia and BMI status

The sociodemographic characteristics, categorized by BMI status and Hb levels, are illustrated in Table 3. Overall, a higher percentage of underweight (12.6%) and anemic (23.7%)

individuals were observed among students aged 25 and above, while obesity (22.5%) was more prevalent among the students aged below 24 years.

**Table 2** Prevalence of anaemia and BMI status of study population (n=341).

| Characteristics | Frequency (%)[a] |
|---|---|
| Non-anemic | 263 (77.1%) |
| Anemic | 78 (22.9%) |
| Severe anemia | 1 (0.3%) |
| Moderate anemia | 35 (10.3%) |
| Mild anemia | 42 (12.3%) |
| Normal BMI | 170 (49.9%) |
| Abnormal BMI | 171 (50.1%) |
| Underweight | 44 (12.9%) |
| Overweight | 59 (17.3%) |
| Obese | 68 (19.9%) |

*Mild anemia: Hb 11–11.9 g/dL in women and 11–12.9 g/dL in men; moderate anemia: Hb 8–10.9 g/dL; severe anemia: Hb < 8 g/dL; BMI, body mass index; underweight: BMI < 18.5 kg/m$^2$; overweight: BMI 23.0-24.9 kg/m$^2$; obese: BMI ≥ 25.0 kg/m$^2$, [a] Unless stated otherwise*

Moreover, anemia was found to be associated with gender, and being female had a significantly higher chance of being affected by anemia (35%, $p < 0.001$). Additionally, an association between family income and BMI status was observed, where students from higher-income households had a significantly higher chance of being obese (31.5%, $p = 0.003$). Nonetheless, no significant association was found between ethnicity, religion, and place of residence and BMI categories or anemia status (Table 3).

The results obtained from the analysis of lifestyle factors in relation to anemia and abnormal BMI revealed that irregular exercise ($p = 0.016$), exercising for less than 20 minutes per session ($p = 0.010$), and consuming green leafy vegetables fewer than four times per week ($p = 0.034$) were significantly associated with anemia. Although students who walked or cycled, slept less than six hours, followed non-vegetarian diets, consumed two or more extra meals per day, ate three or fewer eggs per week, consumed red meat fewer than two times per week, and had a family history of anemia or obesity showed slightly higher anemia prevalence, these associations were not statistically significant (Table 4). Moreover, students with a family history of obesity ($p < 0.001$) and anemia ($p = 0.042$) demonstrated a significantly higher prevalence of overweight and obesity.

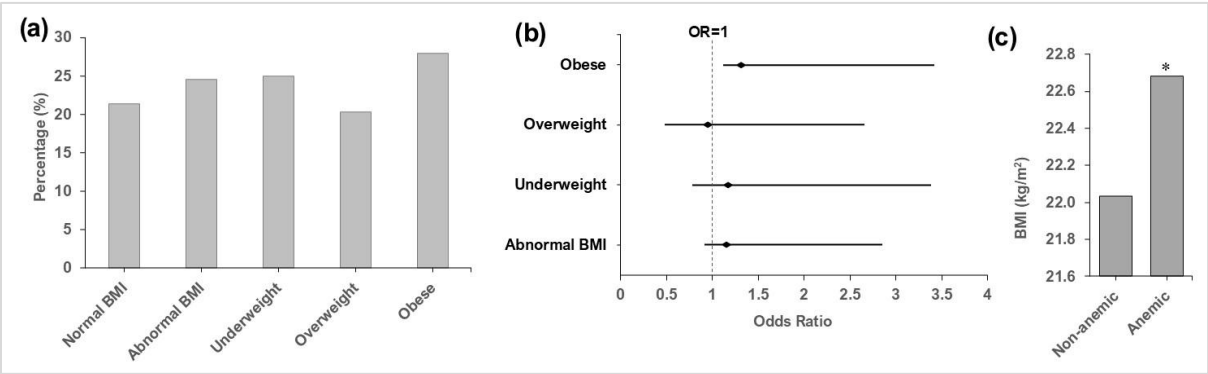



**Figure 1** Prevalence of anemia in different BMI categories

(a), anemia risk in different BMI statuses (b), and the level of BMI between anemia and healthy participants (c) were shown. BMI outside the refence range (OR=1.149, CI=0.778-1.698), underweight (OR=1.171, CI=0.622-2.204), overweight (OR=0.953, CI=0.534-1.699), and obese (OR=1.309, CI=0.814-2.104). *p*-values obtained from an independent t-test. Statistical significance determined as *$p < 0.05$. BMI, Body Mass Index.

**Table 3** Sociodemographic characteristics by BMI status and Hb status

| Characteristics | BMI status (%) | | | | | Hb status (%) | | *p* value |
|---|---|---|---|---|---|---|---|---|
| | Underweight | Normal weight | Overweight | Obese | *p* value | Anemia | Non-anemia | |
| Age | | | | | | | | |
| 20-24 years | 7.9 | 53.0 | 16.6 | 22.5 | 0.429 | 21.9 | 78.1 | 0.689 |
| 25-29 years | 12.6 | 51.6 | 17.9 | 17.9 | | 23.7 | 76.3 | |
| Gender | | | | | | | | |
| Male | 8.9 | 56.1 | 16.3 | 18.7 | 0.728 | 0.8 | 99.2 | <0.001* |
| Female | 11.5 | 50.0 | 17.9 | 20.6 | | 35.3 | 64.7 | |
| Marital status | | | | | | | | |
| Married | 33.3 | 0 | 0 | 66.7 | 0.075 | 66.7 | 33.3 | 0.070 |
| Unmarried | 10.4 | 52.7 | 17.5 | 19.5 | | 22.5 | 77.5 | |
| Ethnicity | | | | | | | | |
| Sinhala | 11.6 | 54.1 | 16.4 | 17.9 | | 23.1 | 76.9 | 0.906 |
| Tamil | 5.7 | 52.8 | 20.8 | 20.8 | 0.063 | 20.8 | 79.2 | |
| Muslim | 10.0 | 25.0 | 20.0 | 45.0 | | 25.0 | 75.0 | |
| Religion | | | | | | | | |
| Buddhist | 11.8 | 54.2 | 16.0 | 17.9 | | 22.9 | 77.1 | 0.873 |
| Hindu | 6.7 | 53.3 | 17.8 | 22.2 | 0.070 | 24.4 | 75.6 | |
| Muslim | 10.0 | 25.0 | 20.0 | 45.0 | | 25.0 | 75.0 | |
| Christian | 0 | 50.0 | 35.7 | 14.3 | | 14.3 | 85.7 | |
| Family monthly income | | | | | | | | |
| < 50,000 LKR | 11.2 | 57.1 | 17.2 | 14.6 | 0.003* | 23.6 | 76.4 | 0.637 |
| > 50,000 LKR | 9.3 | 41.7 | 17.6 | 31.5 | | 21.3 | 78.7 | |
| Residence | | | | | | | | |
| Boarding place | 9.1 | 53.1 | 16.8 | 21.0 | 0.870 | 19.6 | 80.4 | 0.218 |
| Hostel | 11.6 | 51.5 | 17.7 | 19.2 | | 25.3 | 74.7 | |

*p-values* obtained from Chi-squared tests. Statistical significance determined as $*p < 0.05$.

Students with irregular exercise habits, shorter sleep duration, vegetarian diets, irregular meal patterns, and low intake of eggs, red meat, and green leafy vegetables were more likely to be underweight. On the other hand, obese students reported engaging in regular exercise exceeding 20 minutes, using alternative modes of transport, following vegetarian diets, having irregular meal patterns, and consuming more than two additional meals per day. However, these factors were not statistically significantly associated with underweight or obesity (Table 4).

**Table 4** Influence of dietary and lifestyle factors on BMI and Hb status

| Characteristics | BMI status (%) | | | | | Hb status (%) | | |
|---|---|---|---|---|---|---|---|---|
| | Under weight | Normal weight | Over weight | Obese | *p* value | Anemia | Non-anemia | *p* value |
| Exercise | | | | | | | | |
| Regular | 3.8 | 53.8 | 11.5 | 30.8 | 0.333 | 3.8 | 96.2 | 0.016* |
| Irregular | 11.1 | 52.1 | 17.8 | 19.0 | | 24.4 | 75.6 | |
| Exercise ≥20 min | | | | | | | | |
| Yes | 7.5 | 50.9 | 17.9 | 23.6 | 0.489 | 14.2 | 85.8 | 0.010* |
| No | 11.9 | 52.8 | 17.0 | 18.3 | | 26.8 | 73.2 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Travelling mode | | | | | | | | |
| Walking/cycling | 10.3 | 54.8 | 16.2 | 18.8 | 0.291 | 23.5 | 76.5 | 0.567 |
| Others | 11.6 | 42.0 | 21.7 | 24.6 | | 20.3 | 79.7 | |
| Sleeping ≥6h | | | | | | | | |
| Yes | 9.2 | 54.6 | 16.4 | 19.7 | 0.826 | 21.7 | 78.3 | 0.646 |
| No | 11.6 | 50.3 | 18.0 | 20.1 | | 23.8 | 76.2 | |
| Dietary pattern | | | | | | | | |
| Vegetarian | 13.3 | 40.0 | 20.0 | 26.7 | 0.805 | 13.3 | 86.7 | 0.535 |
| Non-vegetarian | 10.4 | 52.8 | 17.2 | 19.6 | | 23.3 | 76.7 | |
| Meal pattern | | | | | | | | |
| Regular | 8.8 | 60.2 | 12.4 | 18.6 | 0.169 | 23.0 | 77.0 | 0.967 |
| Irregular | 11.4 | 48.2 | 19.7 | 20.6 | | 22.8 | 77.2 | |
| Breakfast pattern | | | | | | | | |
| Regular | 12.1 | 51.5 | 15.8 | 20.6 | 0.734 | 22.4 | 77.6 | 0.848 |
| Irregular | 9.1 | 52.8 | 18.8 | 19.3 | | 23.3 | 76.7 | |
| Extra meals | | | | | | | | |
| Yes | 13.7 | 49.3 | 17.1 | 19.9 | 0.424 | 24.0 | 76.0 | 0.676 |
| No | 8.2 | 54.4 | 17.4 | 20.0 | | 22.1 | 77.9 | |
| Number of extra meals/day | | | | | | | | |
| ≥ 2 meals | 16.7 | 39.4 | 21.2 | 22.7 | 0.357 | 24.2 | 75.8 | 0.941 |
| < 2 meals | 10.1 | 55.1 | 15.9 | 18.8 | | 21.7 | 78.3 | |
| None | 8.7 | 55.3 | 16.5 | 19.4 | | 22.8 | 77.2 | |
| Consumption of Eggs/week | | | | | | | | |
| ≤ 3 eggs | 11.2 | 53.9 | 17.0 | 17.8 | 0.776 | 23.7 | 76.3 | 0.647 |
| > 3 eggs | 8.2 | 49.4 | 17.6 | 24.7 | | 22.4 | 77.6 | |
| Never | 13.3 | 40.0 | 20.0 | 26.7 | | 13.3 | 86.7 | |
| Consumption of red meat | | | | | | | | |
| Yes | 9.7 | 53.4 | 16.5 | 20.3 | 0.798 | 22.5 | 77.5 | 0.784 |
| No | 12.4 | 49.5 | 19.0 | 19.0 | | 23.8 | 76.2 | |
| Consumption of red meat/week | | | | | | | | |
| < 2 meals | 9.4 | 51.2 | 18.1 | 21.3 | 0.588 | 27.5 | 72.5 | 0.145 |
| > 2 meals | 6.6 | 56.6 | 15.8 | 21.1 | | 17.1 | 82.9 | |
| Never | 15.2 | 50.5 | 17.1 | 17.1 | | 20.0 | 80.0 | |
| Consumption of green leaves/week | | | | | | | | |
| ≥ 4 times | 5.9 | 45.6 | 19.1 | 29.4 | 0.092 | 13.2 | 86.8 | 0.034* |
| < 4 times | 11.7 | 53.8 | 16.8 | 17.6 | | 25.3 | 74.7 | |
| Consumption of Junk foods | | | | | | | | |
| Every day | 10.2 | 49.2 | 16.9 | 23.7 | 0.110 | 22.0 | 78.0 | 0.912 |
| Once a week | 10.0 | 57.3 | 9.1 | 23.6 | | 21.8 | 78.2 | |
| Not so often | 11.0 | 50.0 | 22.7 | 16.3 | | 23.8 | 76.2 | |
| Consumption of soft drinks | | | | | | | | |
| Everyday | 3.2 | 64.5 | 9.7 | 22.6 | 0.422 | 16.1 | 83.9 | 0.228 |
| Once a week | 13.4 | 53.5 | 16.2 | 16.9 | | 20.4 | 79.6 | |
| Not so often | 9.3 | 48.1 | 19.8 | 22.8 | | 25.3 | 74.7 | |
| Never | 16.7 | 66.7 | 16.7 | 0 | | 50.0 | 50.0 | |
| Family history of anemia | | | | | | | | |
| Yes | 13.6 | 27.3 | 18.2 | 40.9 | 0.042* | 27.3 | 72.7 | 0.612 |
| No | 10.3 | 53.9 | 17.2 | 18.5 | | 22.6 | 77.4 | |
| Family history of Obesity | | | | | | | | |
| Yes | 3.3 | 30.0 | 23.3 | 43.3 | <0.001* | 31.7 | 68.3 | 0.074 |
| No | 12.1 | 56.9 | 16.0 | 14.9 | | 21.0 | 79.0 | |

*p-values obtained from Chi-squared tests. Statistical significance determined as *p < 0.05.*

**Discussion**

Higher BMI and obesity are associated with metabolic complications and non-communicable diseases, and a possible link with anemia has also been suggested; however, the exact nature of this relationship remains controversial [24, 25]. The present study assessed the prevalence of anemia in relation to BMI status and its associated factors among AHS undergraduates at a leading university in Sri Lanka. Overall, 22.9% of subjects with anemia and 37.2% of subjects with obesity/overweight were found in this study. Furthermore, female sex, irregular exercise, exercise less than 20 minutes, consumption of green leaves less than four times a week, were associated with anemia, while lower family income and family history of obesity were associated with abnormal BMI status.

In the present study, anemia was more prevalent (35.3%) among females, and these findings were closely aligned with the previous studies. The WHO reported that the anemic prevalence in Sri Lanka and other Asian countries were 31.6% and 33%, respectively. Furthermore, a study done in 2011 in Sri Lanka indicated that the prevalence of anemia among non-pregnant females aged between 20 and 29 years was 32% [26].

The prevalence of obesity in the world has increased by 6% between 1975 and 2014, reaching 38% by 2023, with developing nations facing the most significant increase [27, 28]. In the present study, over half of the students were found to have BMI outside the reference range: 12.9% of subjects were underweight, 17.3% of subjects were overweight, and 19.9% of subjects were obese. Whereas the previous national survey carried out in Sri Lanka in 2006 indicated that 25% were overweight and 9.2% were obese among the adult population [29], while another study done by Somasundaram et al. (2019) revealed that 7.7% were underweight, 34.3% were overweight, and 31.2% were obese among urban Sri Lankan adults [30]. In our study, there was a slight increase in the prevalence of underweight and a decline in the prevalence of overweight and obesity when compared to previous studies.

In the present study, significant associations were found between anemia and irregular physical activity, exercised for less than 20 minutes, and consumption of green leafy vegetables fewer than four times per week. Evidence from both male and female populations suggests that regular exercise enhances the body's oxygen-carrying capacity by increasing total Hb and red cell mass due to bone marrow stimulation, an improved microenvironment, hormone and cytokine-driven acceleration of erythropoiesis [31]. Furthermore, essential micronutrients, including iron and vitamins, are rich in green leafy vegetables, and insufficient consumption of these vegetables can be associated with anemia [32]. Studies have also reported that children who consume fewer plant-based foods and green leafy vegetables are more likely to be anemic [29, 33, 34].

Although the link between BMI and anemia is continuously under investigation, the exact connection remains a subject of debate. Some studies have suggested that an increase in BMI is associated with higher blood Hb levels and a reduced risk of anemia. Conversely, others

indicated that the prevalence of anemia among obese or overweight women was higher due to inflammation caused by obesity [24, 35, 36]. This study revealed a significant increase in BMI among anemic students, although no significant association between BMI category and anemia prevalence was found. Moreover, individuals with abnormal BMI were found to be anemic, with obese individuals demonstrating a 6.59% higher prevalence and a 1.31 times higher risk ratio. Nonetheless, the risk of anemia among the overweight was slightly lower than that of students with normal BMI. Previous reports indicated that obesity has been associated with anemia by lowering dietary iron uptake from the duodenum, with lower serum iron concentrations linked to higher BMI. As a result, these discrepancies in findings could be attributed to obesity-related inflammation and cytokine production in obese individuals [37, 38]. However, the present study has several limitations, which limit the conclusion that BMI, irregular exercise, or poor dietary habits are the direct causes of anemia. First, it was performed in an institution, which limits the generalizability of the findings. Second, the sample size of the population and gender-biased ratio limit the association of the study, and third, the cross-sectional nature of the study limits the associations of the essential contributors to anemia and obesity/overweight. Fourth, the types of anemia were not studied due to limited resources.

## Conclusion

The prevalence of anemia and BMI outside the reference range among AHS undergraduates in Sri Lanka is high and associated primarily with irregular exercise, poor dietary habits, and a family history of the conditions. Although a higher prevalence of anemia and an increased risk ratio were observed in obese students, further studies are recommended to further understand the relationship between obesity and anemia.

## List of abbreviations

BMI - Body Mass Index
AHS - Allied Health Sciences
UoJ - University of Jaffna
Hb - Hemoglobin

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## Ethical considerations

This study was conducted after obtaining approval from the Ethics Review Committee of the Faculty of Medicine, University of Jaffna, Sri Lanka (approval granted at the 136th meeting held on 23rd June 2022).

## Declaration of conflicting interest

The authors declare that they have no conflict of interest.

## Author contributions

KR and SS conceived the idea and design of the methodology. SAOUS, SHH, TMBST, and KR collected data and did laboratory experiments. SAOUS, SHH, TMBST, SS, SMR, and KR analyzed the data. KR supervised the data analysis, interpreted the statistical analysis, created tables and figures, and drafted the manuscript. SS and KR reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Original Research

# Medication Adherence and Associated Factors among Diabetic Patients Attending the Clinic at a Tertiary Hospital in Northern Sri Lanka

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## Abstract

Diabetes mellitus is one of the most important chronic diseases. The prevalence rate of diabetes is increasing worldwide, and it is rapidly rising in Sri Lanka. Uncontrolled diabetes leads to the development of complications, which will increase morbidity and mortality. Medication non-adherence is one reason for uncontrolled diabetes. Therefore, objective of the study was determination of adherence to oral hypoglycemic drugs among patients with diabetes, and the factors associated with adherence and the barriers to adherence, at the Teaching Hospital, Jaffna, in Northern Sri Lanka. This was a hospital-based cross-sectional study conducted among 420 patients with diabetes mellitus attending the Teaching Hospital in Jaffna, Sri Lanka. A pretested, interviewer-administered questionnaire and a data extraction form were used to collect data on medication adherence and associated factors. Data were analysed using SPSS, version 21.0. The Chi-square test was used to assess the association between variables. A *p*-value less than 0.05 was considered significant. The response rate was 99% (n=416). The majority of the patients (78.1%) adhered to their therapy. The most reported reasons for non-adherence to oral anti-diabetic drugs were lack of reminders, followed by misplaced medication, and being busy or late for work. Age, occupation, family support, frequency of blood glucose checks, regular clinic visits, total number of drugs, number of oral hypoglycemic agents, and total weekly doses of oral hypoglycemic agents were significantly associated with adherence. Most patients reported good adherence to diabetic medication at the Teaching Hospital in Jaffna. More attention should be taken among non-adherent patients by considering the factors associated with non-adherence and providing proper counselling on medication usage to them.

***Key Words:*** *Associated factors, Diabetic patients, Medication adherence*

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## Introduction

Diabetes mellitus (DM) is caused by either diminished pancreatic insulin secretion or the emergence of insulin resistance, which impairs the body's ability to use insulin [1]. The prevalence of adult-onset diabetes was 10.5% worldwide [2]. In Sri Lanka, 23 % of adults have diabetes mellitus [3], which is greater than the prevalence of diabetes worldwide.

The treatment guidelines for diabetes are based on the type of diabetes. The mainstay treatment for type 1 diabetes is insulin. For the treatment of type 2 diabetes, insulin and various hypoglycemic agents are used [4]. Glycemic control in patients with diabetes reduces diabetes-related complications [5,6]. Adherence to medication is associated with a controlled blood glucose level [7]. Nonadherence to glucose-lowering drugs is associated with higher glycated haemoglobin [8].

The World Health Organisation (WHO) defined medication adherence as “The extent to which a person’s behaviour in taking medication, following a diet, and executing lifestyle changes, corresponds with agreed recommendations from a healthcare provider” [9]. Adherence problems are common in the management of diabetes [10]. Medication non-adherence may begin at any stage of treatment, including not filling the prescription regularly, non-persistence with the therapy, incorrect drug or dose administration, or skipping doses.

Diabetic patients who were non-adherent to medication have a greater risk of developing cardiovascular complications [11,12]. The medication non-adherence affects mainly the older adult groups, as they are more susceptible to adverse health effects due to the change in their pharmacokinetics and pharmacodynamics [13].

A gold-standard method for measuring drug adherence is not available [14]. Drug adherence can be measured directly or indirectly. Direct methods are relatively accurate and objective, including biomedical measurements and direct observations. Self-reported questionnaires, electronic monitoring devices, and pill counts are examples of indirect methods [15]. Because self-report questionnaires are affordable and easy to administer, they are frequently used [16].

Medication non-adherence among diabetic patients could develop different complications and affect patients’ quality of life. There are limited studies on medication adherence of diabetes patients in Northern Sri Lanka. It is essential to determine the factors associated with non-adherence and barriers to adherence among diabetic patients. Such a study will help elaborate on strategies to improve medication adherence among health care professionals. Self-reported questionnaires were used to determine adherence in other countries. However, such a questionnaire should be used only after validation to obtain reliable results. Self-reported missed doses by patients is one method used to measure medication adherence.

This study aims to evaluate medication adherence, related factors, and barriers to adherence among diabetic patients at the Jaffna Teaching Hospital in Northern Sri Lanka.

## Methods

### Study setting

A hospital-based cross-sectional study was conducted among patients with diabetes mellitus attending the Diabetes clinic at Teaching Hospital, Jaffna.

### Study population

Adult patients with type II diabetes mellitus who had been taking oral anti diabetic drugs for at least the past three months were selected in this study. Patients who could not communicate properly and had insulin therapy were excluded from the study. The study was conducted from November 2020 to November 2021.

### Sample size

The sample size was determined using the following formula: $N=Z^2 p (1-p)/d^2$. An anticipated population proportion of 0.565 was used, based on a study conducted at a tertiary hospital in India that reported patients' adherence to diabetic medication at 56.5% [17]. According to this study the estimated sample size was 378. A 10% non-response rate was added, yielding a total sample size of 420.

### Sampling technique

Approximately 450 patients attend the diabetes clinic per month. Therefore, all patients visiting the clinic in a month were included in this study without any sampling technique.

### Study instruments

Data was gathered using a data extraction form and a pretested, interviewer-administered questionnaire. Information on medication adherence, barriers to adherence, socio-demographic factors, and patient-, disease-, and therapy-related factors was collected using an interviewer-administered questionnaire. The data extraction form was used to collect information from individual patients' clinic books. This information included prescribed oral hypoglycemic agents and doses, dosing frequency, and the total number of prescribed medications.

### Data collection

The data were collected through a face-to-face interview conducted by the researcher with the help of a trained data collector. The purpose of the research was explained to the patients, and informed consent was obtained. Patient privacy was maintained during data collection by interviewing patients in a separate place from other patients.

### Data analysis

The total number of doses missed by the patient during the last week was obtained from the adherence questionnaire. The adherence percentage was calculated using the following equations.

Adherence = (Number of doses recommended – number of doses missed)/(Total number of doses recommended) * 100%

According to the value obtained from the above equation, values ≥ 80% were considered good adherence to the medication, and values < 80% were considered poor adherence [18].

The Chi-square test was employed to evaluate the relationship between selected factors and

medication adherence using Statistical Package for the Social Sciences (SPSS) software, version 21.0. Statistical significance was defined as a *p*-value < 0.05.

## Results

Among participants, the response rate was 99 % (n=416).

### Socio-demographic characteristics of patients

Table 1 depicts the participants' socio-demographic characteristics. Most participants (70.9%, n=295) were aged 18-64 years. The mean age of the patients was 57.69 ±12.2 years. Most of them (83.2%, n=346) were married. Two-thirds of the participants (68.8%, n=286) were female, and over half (57.9%, n=241) had completed secondary/tertiary education. The majority of the participants (71.4%, n=297) were unemployed.

**Table 1** Distribution of socio-demographic factors of Diabetic patients (N=416)

| Socio-demographic factors | Categories | Frequency (Percentage) |
|---|---|---|
| Age (in years) | 18-64 | 295 (70.9%) |
| | >64 | 121(29.1%) |
| Gender | Male | 130 (31.2%) |
| | Female | 286 (68.8%) |
| Marital status | Single | 21 (05%) |
| | Married | 346 (83.2%) |
| | Divorce/ Widowed | 49 (11.8%) |
| Level of education | No formal education | 40 (9.6%) |
| | Primary | 35 (32.5%) |
| | Secondary/ Tertiary | 241(57.9%) |
| Occupation | Employed | 119 (28.6%) |
| | Unemployed | 297 (71.4%) |

### Medication adherence of diabetic patients

The majority of participants (78.1%) adhered well to their therapy.

### Reasons for medication non-adherence among the study participants

The most common reasons for not taking oral hypoglycemic agents (OHA) were lack of reminders (89.2%, n=140), misplaced medication (56.2%, n=89), and being busy or late for work (34.4%, n=54), as shown in Table 2.

**Table 2** Reasons for medication non-adherence of Diabetic patients (N=157)

| Reasons* | Frequency (Percentage) |
|---|---|
| Busy or late for work | 54 (34.4%) |
| Being away on weekends/vacation | 17 (10.8%) |
| Lack of reminders | 140 (89.2%) |
| Misplacing the medication | 89 (56.2%) |
| Finishing the pills | 36 (22.9%) |
| Having too many medications | 30 (19.1%) |
| Lack of money to refill the medication | 14 (8.9%) |
| Being outside and forgetting to take the medication | 41 (26.1%) |

** Multiple options*

### Factors associated with medication adherence

Table 3 shows the association between the sociodemographic factors and medication adherence. Sociodemographic factors, such as age (*p*=0.046) and occupation (*p*=0.037), were significantly associated with medication adherence.

According to Table 5, Patient-related factors, such as the frequency of blood glucose checkups (*p*=0.003), family support (*p*=0.016), and regular clinic visits (*p*=0.004), were significantly

associated with medication adherence. According to Table 6, all therapy-related factors, such as the total number of drugs ($p$=0.013), the number of OHA drugs ($p$>0.001), and the total number of OHA doses per week ($p$>0.001), were significantly associated with medication adherence.

**Table 3** Association between sociodemographic factors and medication adherence among Diabetic patients

| Factor | Category | Medication adherence level | | Total | Chi-square test *p* value |
|---|---|---|---|---|---|
| | | Good adherence N (%) | Poor adherence N (%) | | |
| Age (years) | 18-64 | 228 (77.3%) | 67 (22.7%) | 295 (100%) | **0.046** |
| | ≥65 | 104 (86.0%) | 17 (14.0%) | 121 (100%) | |
| Gender | Male | 99 (76.2%) | 31 (23.8%) | 130 (100%) | 0.512 |
| | Female | 226 (79.0%) | 60 (21.0%) | 286 (100%) | |
| Marital status | Single | 17 (81.0%) | 4 (19.0%) | 21 (100%) | 0.860 |
| | Married | 271 (78.3%) | 75 (21.7%) | 346 (100%) | |
| | Divorced/Widowed | 37 (75.5%) | 12 (24.5%) | 49 (100%) | |
| Level of education | No formal education | 27 (67.5%) | 13 (32.5%) | 40 (100%) | 0.135 |
| | Primary | 111 (82.2%) | 24 (17.8%) | 135 (100%) | |
| | Secondary/Tertiary | 187 (77.6%) | 54 (22.4%) | 241 (100%) | |
| Financial support | Adequate | 256 (77.6%) | 74 (22.4%) | 330 (100%) | 0.596 |
| | Not adequate | 69 (80.2%) | 17 (19.8%) | 86 (100%) | |
| Occupation | Employed | 85(71.4%) | 34 (28.6%) | 119 (100%) | **0.037** |
| | Unemployed | 240 (80.8%) | 57 (19.2%) | 297 (100%) | |

**Table 4** Association between disease-related factors and medication adherence among Diabetic patients

| Factor | Category | Medication adherence level | | Total | Chi-square test *p* value |
|---|---|---|---|---|---|
| | | Good adherence N (%) | Poor adherence N (%) | | |
| Duration of treatment of DM | ≤ 10 years | 211 (77.6%) | 61 (22.4%) | 272 (100%) | 0.708 |
| | >1 years | 114 (79.2%) | 30 (20.8%) | 144 (100%) | |
| Having co-morbidities | Yes | 206 (79.2%) | 54 (20.8%) | 260 (100%) | 0.481 |
| | No | 119 (76.3%) | 37 (23.7%) | 156 (100%) | |
| Having DM complications | Yes | 226 (77.7%) | 65 (22.3%) | 291 (100%) | 0.728 |
| | No | 99 (79.2%) | 26 (20.8%) | 125 (100%) | |
| Family history of DM | Yes | 174 (78.7%) | 47 (21.3%) | 221 (100%) | 0.749 |
| | No | 151 (77.4%) | 44 (22.6%) | 195 (100%) | |

**Table 5** Association between patient-related factors and medication adherence among Diabetic patients

| Factor | Category | Medication adherence level | | Total | Chi-square/Fisher's exact tests *p* value |
|---|---|---|---|---|---|
| | | Good adherence N (%) | Poor adherence N (%) | | |
| Family support | Yes | 123 (84.8%) | 22 (15.2%) | 145 (100%) | **0.016** |
| | No | 202 (74.5%) | 69 (25.5%) | 271 (100%) | |
| Frequency of blood glucose level checkup | Regularly | 83 (88.3%) | 11 (11.7%) | 94 (100%) | **0.003** |
| | Sometimes | 184 (78.0%) | 52 (22.0%) | 236 (100%) | |
| | Rarely | 58 (67.4%) | 28 (32.6%) | 86 (100%) | |
| Regular clinic visit | Yes | 224 (82.4%) | 48 (17.6%) | 272 (100%) | **0.004** |
| | No | 101 (70.1%) | 43 (29.9%) | 144 (100%) | |
| Alcohol consumption | Yes | 18 (75.0%) | 06 (25.0%) | 24 (100%) | 0.703 |
| | No | 307 (78.3%) | 85 (21.7%) | 392 (100%) | |
| Smoking | Yes | 8 (66.7%) | 4 (33.3%) | 12 (100%) | 0.477 |
| | No | 317 (78.5%) | 87 (21.5%) | 404 (100%) | |

**Table 6** Association between therapy-related factors and medication adherence among Diabetic patients

| Factor | Category | Medication adherence level | | Total | Chi-square test *p* value |
|---|---|---|---|---|---|
| | | Good adherence N (%) | Poor adherence N (%) | | |
| Total number of drugs | < 5 drugs | 174 (73.7%) | 62 (26.3%) | 236 (100%) | **0.013** |
| | ≥ 5 drugs | 151 (83.9%) | 29 (16.1%) | 180 (100%) | |
| No. of OHA drugs | One | 149 (67.42%) | 72 (32.58%) | 221 (100%) | **< 0.001** |
| | More than one | 176 (90.26%) | 19 (9.74%) | 195 (100%) | |
| Total number of OHA doses per week | ≤ 14 | 79 (54.5%) | 66 (45.5%) | 145 (100%) | **< 0.001** |
| | >14 | 246 (90.7%) | 25 (9.3%) | 271 (100%) | |

None of the disease-related factors showed a significant association with medication adherence, as depicted in Table 4.

## Discussion

This study examined medication adherence to oral hypoglycemic drugs, factors associated with adherence, and barriers to adherence among patients with diabetes at the Teaching Hospital in Jaffna, in the Northern Province of Sri Lanka. More than three-fourths of patients (78.1%) had good medication adherence in this study. Medication adherence to diabetic medications was low to moderate in the Asian region [19]. And it was 26% to 97% in low- and middle-income counties [20]. Medication adherence to anti-diabetic medications was 61% in South India [21], 79.5% in North India [22], 34.14% in eastern India [23], 82% in Pakistan [24], 53.7% in Bangladesh [25], 61% in Nepal [26], 17.7% in Iran [27], and 65.8% in Malaysia [28]. Even though medication adherence in our study was higher than in other studies, the inconsistent adherence rates are due to differences in patients' socio-demographic characteristics, the methods used to measure

adherence, and study settings (e.g., community vs hospital).

Lack of reminder, misplaced medication, and being busy or late for work were the reported reasons for non-adherence in this study. The most commonly reported reason for poor adherence is forgetfulness, as reported in other studies [29,30,31]. Busy work schedules, misplaced medication, perceived side effects, lack of finances, and being away from home were also reported as reasons for non-adherence in other studies [31, 32]. By considering the reasons for non-adherence in our study, proper counselling should be provided by introducing appropriate reminder systems for drug use, such as encouraging patients to keep medication in a pill box, using mobile apps or mobile messages, and reminding family or caregivers at medication-taking time.

Among sociodemographic factors, only patients' age and occupation were significantly associated with medication adherence. However, gender, educational level, financial support, and marital status showed no association with medication adherence. A study done at the tertiary care hospital in eastern India also reported that gender, marital status, and educational status failed to show an association with medication adherence [23]. Older adults were more likely to adhere to the treatment in our study. A study conducted at a primary health center in Spain found that elders adhered to medication more than others [30]. A Singaporean study conducted among community-dwelling adults also reported higher adherence among older adults than young adults [33]. This could be because most participants are retired at 60 and may have had more time to adhere to the treatment regimen properly. Gender did not show a significant association with medication adherence in our study. In a study of patients attending pharmacies in Germany, adherence to antidiabetic agents did not show a gender pattern [34]. However, in a study conducted among patients at a tertiary hospital in eastern India [21] and in a hospital-based cross-sectional study in Ethiopia [35], females showed lower adherence than males. Even though patients without formal education had lower adherence than their counterparts, education level was not associated with adherence. Lower adherence among patients with low educational levels was also reported in studies conducted in a diabetic clinic in India [36] and among outpatients at a tertiary hospital in Nepal [37]. Unemployed patients have more adherence than others in our study. Homemakers were more adherent among patients at the Teaching Hospital of Ethiopia [38]. In our study, homemakers were included in the unemployed category. Since homemakers have adequate time to follow the treatment, they showed high adherence.

A systematic review conducted in Asian studies reported that patient-related factors were associated with medication adherence [19]. In our study, Family support in managing DM is significantly associated with medication adherence. Reminding them to take medication or helping them organise medication in boxes, etc., are considered family support and could positively impact medication adherence [39,40]. Regular blood glucose monitoring was also significantly associated with medication adherence in our study. Patients who regularly

checked their glucose levels showed better medication adherence. Other studies also observed an association between blood glucose monitoring and adherence [17, 31, 40]. In our study, having regular clinic visits was associated with medication adherence. This finding was supported by a study done at primary health centers in Saudi Arabia [41]. During regular clinic visits, patients meet healthcare professionals and receive advice on medication use, improving their adherence. Alcohol consumption and smoking had no association with adherence in our study. Alcohol consumption was significantly influenced by non-adherence in a study conducted in a regional hospital in Cameroon [29]. Alcohol usage and smoking were significantly associated with adherence in a study done at a tertiary care hospital in eastern India [23].

There was an association between medication adherence and the number of medicines, OHA drugs, and OHA doses under therapy-related factors. Polypharmacy is defined as the use of more than five drugs, excluding antibiotics, injectable medications, and over-the-counter medicines. Polypharmacy increases the risk of nonadherence [42]. According to a systematic review, polypharmacy was negatively associated with medication adherence in many studies, but in some studies it was not [19]. Surprisingly, in our research, polypharmacy significantly influenced adherence, consistent with previous studies in a nationwide claim-based database in Japan [43] and a prospective cohort study in Germany [44]. Taking more medications for a longer duration of time could have become a habitual action for patients, which could be the reason for good adherence among patients on polypharmacy. Patients who have poor glycemic control were prescribed more medications to control blood sugar and reduce complications [45]. Patients would also be advised by healthcare professionals about the development of diabetes complications in uncontrolled glycemic status, so that patients can pay more attention to adhering to therapy in such conditions.

None of the disease-related factors, such as duration of diabetes treatment, comorbidities, diabetes-related complications, and family history, showed a significant association with medication adherence in this study. Diabetes duration was not associated with adherence in a study conducted in Bangladesh [46]. However, a study done in eastern India reported that having diabetic complications, having comorbidities, and having a family history of diabetes showed an association with medication adherence [23].

It is crucial to ensure medication adherence among non-adherents. Especially adults and employed patients, patients with less family support and those who have irregular clinic visits, need more attention, and they need proper counselling to address the importance of taking medications regularly.

To obtain generalizable results, the same study needs to be conducted in community settings and expanded to other parts of Sri Lanka. The findings would be helpful for improving adherence to antidiabetic medications during patient counselling. Recall bias and social desirability could have affected our findings. Lack of control for confounders is another limitation in this study.

## Conclusion

More than three-fourths of patients with diabetes adhered to oral hypoglycemic therapy in the Teaching Hospital, Jaffna. However, among non-adherents, more attention should be given to improve medication adherence, especially among older adults and employed patients. Further, therapy- and patient-related factors need to be considered to improve medication adherence while counselling is given to non-adherent patients. Since this study was conducted among hospital-based patients, further research in community settings is needed to evaluate medication adherence.

## List of abbreviations

DM - Diabetes mellitus
OHA - Oral hypoglycemic agents

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## Ethical considerations

Ethical approval was obtained from the Ethics Review Committee, Faculty of Medicine, University of Jaffna. Administrative clearance was obtained from the Director and the Consultant Endocrinologist at the Teaching Hospital, Jaffna.

## Declaration of conflicting interest

The authors declare that they have no conflict of interest.

## Author contributions

ST and PADC were involved in the Study conception and design. PADC critically revise the manuscript for intellectual content. ST is responsible for data collection. Both authors have read and approved the final version of the manuscript.

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Original Research

# Effect of Time Delay at Ambient Temperature on Serum Electrolytes and Comparison of Electrolyte Concentrations in Plasma and Serum

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## Abstract

The present study was aimed at investigating the effect of time delay at ambient temperature on laboratory investigation of serum electrolytes and to compare the electrolytes concentrations in plasma and serum. The study was conducted by recruiting 32 healthy individuals of 22-35 years. Whole blood samples (6 mL) were collected from each individual and plasma and serum were separated. Serum was divided into four aliquots. One aliquot was analysed immediately after the separation (baseline value) and other three aliquots were kept for 1, 3 and 5 hours at ambient temperature (28-30 °C). The plasma and serum electrolytes; $Na^+$, $K^+$, $Cl^-$ and $iCa^{2+}$ concentrations were measured by ST-200 Plus Electrolyte Analyzer. There were no statistically significant differences in serum $Na^+$ concentrations between serum analysed immediately after separation (142.60±2.31 mmol/L) and serum kept for 1 hour at ambient temperature (142.63±2.27 mmol/L) ($p$>0.05). Similarly, no statistically significant differences were observed in serum $Cl^-$ concentrations between serum analysed immediately after separation (101.27±1.46 mmol/L) and serum stored at ambient temperature for 1 hour (101.43±1.48 mmol/L) and 3 hours (101.55±1.39 mmol/L) ($p$>0.05). Comparison of baseline plasma and serum electrolyte concentrations demonstrated no statistically significant difference in $Na^+$ concentrations between plasma (142.75±2.23 mmol/L) and serum analysed immediately after separation (142.60±2.31 mmol/L) ($p$>0.05). However, a statistically significant difference was observed between plasma and serum $K^+$ concentrations, with plasma showing 4.09±0.31 mmol/L and serum analysed immediately after separation showing 4.21±0.31 mmol/L ($p$<0.05). Likewise, a statistically significant difference was observed between plasma and serum $iCa^{2+}$ concentrations, where plasma showed 1.25±0.03 mmol/L and serum analysed immediately after separation showed 1.27±0.03 mmol/L ($p$<0.05). No statistically significant difference was observed between plasma (101.54±1.33 mmol/L) and serum analysed immediately after separation (101.27±1.46 mmol/L) in relation to $Cl^-$ concentrations ($p$>0.05). Immediately separated plasma following blood collection can be suggested as the sample of choice for the determination of $Na^+$, $K^+$, $Cl^-$, and $iCa^{2+}$ when multiple electrolyte parameters are analysed from a single sample.

***Keywords***: *Ambient temperature, Electrolytes, Plasma, Serum*

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## Introduction

Electrolytes are minerals that carry positive or negative electrical charges when dissolved in aqueous media. They exist as cations or anions and include acids, bases, and salts [1]. The major electrolytes present in the human body include sodium, potassium, chloride, calcium, phosphate, magnesium, bicarbonate, and sulfate [2]. These electrolytes are essential for maintaining normal physiological functions and play critical roles in acid–base balance, fluid balance, nerve impulse transmission, and muscle contraction.

Sodium is the predominant cation in extracellular fluid, whereas potassium is the principal intracellular cation. Chloride is the major extracellular anion. Sodium and potassium are important in maintaining osmotic pressure in extracellular and intracellular compartments, respectively. Potassium is also involved in cardiac muscle contraction and regulation of hydrogen ion concentration. Chloride contributes to maintaining electrical neutrality by moving passively with sodium and in exchange with bicarbonate. Furthermore, chloride plays an essential role in carbon dioxide transport through the chloride shift mechanism and contributes to oxygen exchange. Calcium is a vital structural component of bones and teeth and is involved in blood coagulation, cell division, endocrine regulation, and cardiac function [3,4].

Electrolyte imbalance may result from various conditions, including alcohol consumption, excessive sweating, cirrhosis, diabetes mellitus, kidney diseases, severe burns, trauma, eating disorders, hypertension, congestive heart failure, and thyroid and adrenal gland disorders [5,6]. Disturbances in electrolyte homeostasis can lead to severe and potentially life-threatening complications [2]. Potassium imbalance may adversely affect renal, cardiovascular, muscular, and neurological functions. Severe hypokalemia may result in respiratory compromise and paralysis [7,8]. Hypocalcemia can present with manifestations ranging from asymptomatic conditions to severe complications such as seizures [9,10]. Hypercalcemic crisis is a potentially life-threatening condition that may result in cardiac rhythm abnormalities, hypotension, renal failure, neurological impairment, and musculoskeletal complications including osteoporosis and bone pain [11,12]. Sodium imbalance affects nearly all body cells, particularly those of the central nervous system. Hypernatremia can result in cellular dehydration and brain cell shrinkage, whereas hyponatremia may lead to cerebral edema [13,14].

Numerous studies have investigated the association between serum electrolyte concentrations and diseases such as pancreatic disorders, kidney diseases, sickle cell disease, and COVID-19 [15–17]. However, relatively few studies have focused on the impact of pre-analytical factors on electrolyte determination in medical laboratories.

Accurate measurement of serum electrolyte concentrations is critical for appropriate clinical decision-making and patient management. Pre-analytical variables, including delays in sample processing and analysis, may affect the reliability of electrolyte measurements and consequently influence laboratory reporting.

Therefore, timely processing and analysis of blood samples are essential to ensure accurate electrolyte determination and minimize the risk of clinical misinterpretation arising from inaccurate laboratory results.

Delays in serum/plasma electrolyte analysis due to heavy laboratory workload or prolonged sample transportation from hospital wards and collection centers are common challenges encountered in many hospitals and laboratory settings, particularly in developing countries. Such delays may contribute to preanalytical variations that can potentially influence laboratory results. Although several studies have investigated preanalytical factors affecting electrolyte measurements [18-23], the effect of time delay at ambient temperature on plasma and serum electrolyte concentrations, specifically $Na^+$, $K^+$, $Cl^-$, and $Ca^{2+}$ when requested together in a single laboratory test panel, has not been adequately investigated.

This issue is particularly relevant in tropical countries characterized by warm and humid climatic conditions, where delays in sample processing may have a greater impact on analyte stability. Therefore, understanding the influence of delayed sample processing at ambient temperature on electrolyte concentrations is important to ensure analytical accuracy and reliability in clinical laboratories.

The present study was designed to investigate the effect of delayed processing at ambient temperature on serum electrolyte measurements, specifically $Na^+$, $K^+$, $Cl^-$, and $Ca^{2+}$ concentrations, and to compare electrolyte concentrations between plasma and serum samples. Plasma can be separated immediately following blood collection through centrifugation of anticoagulated blood, whereas serum preparation requires a clotting period before centrifugation. This difference in sample processing time may introduce preanalytical alterations that could influence electrolyte measurements. Therefore, the present study aimed to determine whether delayed serum separation contributes to significant changes in electrolyte concentrations compared with plasma and to evaluate whether differences exist between plasma and serum electrolyte measurements under routine laboratory conditions

## Methods

### Study design

This study was an analytical observational cross-sectional study with laboratory investigations.

### Sample size calculation

The sample size was determined using a one-way analysis of variance (ANOVA) statistical power calculator, considering the study design and expected variability of electrolyte measurements. Potassium was used as the reference analyte for sample size estimation because it yielded the largest required sample size among the electrolytes evaluated, thereby ensuring adequate statistical power for all electrolytes included in the study.

The calculation was performed using the following parameters: number of groups (levels) = 5, maximum expected difference between

group means = 0.44, standard deviation = 0.50 (19), and statistical power = 80% (0.80).

The one-way ANOVA power calculation estimates the required sample size by incorporating the number of study groups, expected effect size derived from the maximum difference between means, variability within the data (standard deviation), and the desired statistical power. Among all electrolytes assessed, potassium generated the highest sample size requirement. Therefore, to ensure sufficient statistical power across all electrolyte analyses, a sample size of 32 samples per electrolyte parameter was selected for the study.

**Study population**

Participants were recruited using the convenient sampling method. Prior to study commencement, all participants were provided with an information sheet outlining the study details, including its objectives, implications, expected outcomes, and sample collection procedures. Written informed consent was obtained before participation. A self-administered questionnaire was used to collect socio-demographic information from the participants.

The study population included 32 healthy individuals (male and female) within 22-35 years. Individuals clinically diagnosed with dehydration, vomiting and other clinical manifestations due to imbalanced serum electrolytes, cirrhosis, diabetes mellitus, kidney diseases, trauma, eating disorders, hypertension, congestive heart failure, thyroid and adrenal gland disorders, pregnant and lactating women, people with burns, people with alcohol consumption, people use any medicine that causes alteration in serum electrolytes concentrations, individuals undergone surgeries and people doing severe exercises with excessive sweating were excluded.

**Collection of blood samples**

Venipuncture was performed by a well-experienced phlebotomist between 9:00 and 9:30 a.m. while adhering to standard safety precautions. No specific patient preparation was required prior to blood collection for serum/plasma electrolyte determination. A total of 6 mL of whole blood was collected from each participant and immediately divided into two aliquots by gentle transfer to avoid haemolysis. Of the collected blood, 1 mL was transferred into a lithium heparin-containing tube for plasma separation, while the remaining 5 mL was transferred into a plain tube without anticoagulant for serum separation.

**Separation of plasma**

The sample aliquot of 1 mL was centrifuged at 3000 rpm for 5 minutes to separate plasma. Plasma was analysed immediately after the separation to determine the baseline electrolytes concentration.

**Separation of serum**

Five milliliter blood sample was left undisturbed at room temperature for 30 minutes by keeping the plain tubes with blood in an upright position in a tube rack. After 30 minutes, the tubes were checked for clot formation. All the samples were observed clotted after 30 minutes. Then, the tubes were gently tapped in order to detach the

clot from the bottom of the tubes. Next, the clotted blood samples in plain tubes were centrifuged at 3000 rpm for 5 minutes. After the centrifugation, tubes were observed for haemolysis and serum was separated.

**The effect of time-delay on serum electrolyte concentration**

Each serum sample was divided into four aliquots each with 200 µL and transferred into four Eppendorf tubes which were properly labeled according to the respective serial numbers. All the Eppendorf tubes were capped tightly and placed in a plastic tube rack. Out of four serum aliquots, aliquot No: 1 was analysed for electrolytes immediately upon serum separation. Aliquot No: 2, 3 and 4 were kept for 1, 3 and 5 hours at ambient temperature (28-30 °C), respectively.

The concentrations of four serum electrolytes i.e., sodium ($Na^+$), potassium ($K^+$), chloride (Cl), and ionised calcium ($iCa^{2+}$) were measured using SENSA CORE ST-200 Plus semi-automated, microprocessor-controlled electrolyte analyzer (India). Direct Ion-selective Electrode (ISE) method was used to determine the serum electrolytes. Before the analysis, the analyzer was calibrated for the serum electrolytes. Following the calibration of the analyzer, two quality control samples (ST-200 (Pro/Plus/CL) reagent pack, India) were used to check the quality of the clinical chemistry assay samples in the analyzer. The plasma and serum samples were analysed after obtaining the results of the quality control samples.

Data analysis was done using IBM Statistical Package for Social Sciences (SPSS) software version 25.0. Paired sample t-test was used to compare the electrolyte concentrations in plasma and serum. One-way repeated measures ANOVA test was used to compare the mean values of electrolyte concentrations in serum samples which kept for 1, 3 and 5 hours at ambient temperature. The Dunnett t (2-sided) test was used to compare the electrolyte concentrations in serum analysed immediately after the separation with serum kept at ambient temperature for 1, 3 and 5 hours. In order to check the individual differences of mean serum electrolyte concentrations at different time intervals, the post-Hoc comparisons using the Tukey HSD (Honest Significant Difference) test was used.

**Results**

**Comparison of electrolyte concentrations in plasma and serum**

Table 1 shows the electrolyte concentrations of plasma and serum kept under the ambient temperature for different time intervals. The electrolyte concentrations of the 32 plasma samples vary as; $Na^+$; 138.10-145.80, $K^+$; 3.52-4.81, $Cl^-$; 98.40-104.00 and $iCa^{2+}$; 1.18-1.32 mmol/L. Similarly, baseline serum electrolyte concentrations vary as $Na^+$; 138.40-146.00, $K^+$; 3.65-4.95, $Cl^-$; 98.30-104.20 and $iCa^{2+}$; 1.21-1.35 mmol/L.

Though there was a reduction in baseline $Na^+$ concentrations in serum compared to plasma it was not statistically significant (*p>0.05*). The serum $K^+$ concentration was higher than the plasma and it was statistically significant (*p=0.000*). Even though the serum $Cl^-$

concentration was less than the plasma it was not statistically significant (*p>0.05*). In relation to $iCa^{2+}$ concentration, there was a significant difference between baseline concentrations of serum and plasma (*p=0.006*). The $iCa^{2+}$ concentration in serum was 0.02 mmol/L higher than plasma.

**Effect of time-delay on serum sodium ion concentration**

Table 1 indicates the Na+ concentrations given by serum samples; immediately after the separation and which kept for 1, 3 and 5 hours at ambient temperature.

**Comparison of sodium ion concentrations in serum**

Sodium ion concentrations were not same in the serum samples kept for 1, 3 and 5 hours at ambient temperature. According to the post-Hoc test there was a significant difference in $Na^+$ concentrations in serum kept for 1 and 3 hours (*p*=0.003). There was a reduction in serum $Na^+$ concentration at 3 hours compared to 1 hour and the difference was 0.39 mmol/L. There was a reduction in $Na^+$ concentration at 5 hours compared to 1 hour. The difference was 0.61 mmol/L and statistically significant (*p*=0.000). There was a reduction in serum $Na^+$ concentration at 5 hours compared to 3 hours and the difference was 0.22 mmol/L. However, it was not statistically significant (*p*>0.05).

According to the Dunnett t (2-sided) test, serum kept for 1 hour showed a higher $Na^+$ concentration compared to the serum analysed immediately after the separation, but it was not statistically significant (*p*>0.05). Three hour kept serum showed a lower $Na^+$ concentration compared to serum analysed immediately after the separation and the difference was 0.36 mmol/L. This difference was statistically significant (*p*=0.005). The same trend was observed between the serum analysed immediately after the separation and kept for 5 hours with the *p*=0.000. The difference of $Na^+$ concentration was 0.58 mmol/L.

**Table 1** Electrolytes concentrations of plasma and serum kept under the ambient temperature for different time intervals.

| Sample | Sodium | Potassium | Chloride | Ionised Calcium |
|---|---|---|---|---|
| Immediately upon plasma separation | 142.75±2.23 | 4.09±0.31 | 101.54±1.33 | 1.25±0.03 |
| Immediately upon serum separation | 142.60±2.31 | 4.21±0.31 | 101.27±1.46 | 1.27±0.03 |
| Serum after 1 hour at ambient temperature | 142.63±2.27 | 4.24±0.30 | 101.43±1.48 | 1.24±0.03 |
| Serum after 3 hours at ambient temperature | 142.24±2.26 | 4.20±0.31 | 101.55±1.39 | 1.12±0.04 |
| Serum after 5 hours at ambient temperature | 142.02±2.15 | 4.20±0.33 | 101.72±1.42 | 1.16±0.08 |

*Note: n = 32, SD = Standard Deviation, Electrolytes levels are given as Mean±SD in mmol/L*

**Effect of time-delay on serum potassium ion concentration**

Table 1 shows $K^+$ concentrations given by serum samples, immediately after the separation and kept for 1, 3 and 5 hours at ambient temperature.

**Comparison of potassium ion concentrations in serum**

There were no statistically significant differences of $K^+$ concentrations in serum, immediately after the separation and kept for 1, 3 and 5 hours at ambient temperature ($p$>0.05).

**Effect of time-delay on serum chloride ion concentration**

The $Cl^-$ concentrations given by serum samples, immediately after the separation and kept for 1, 3 and 5 hours at ambient temperature are given in Table 1.

**Comparison of chloride ion concentrations in serum**

The results showed that serum $Cl^-$ concentrations were increased with time. However, there was no significant difference in $Cl^-$ concentrations between 1 and 3 hours serum samples and 3 and 5 hours serum samples ($p$>0.05). However, there was a 0.29 mmol/L increase in $Cl^-$ concentration at 5 hours compared to 1 hour and it was statistically significant ($p$=0.017). However, there were no significant differences in $Cl^-$ concentrations in 1 and 3 hours kept serum samples compared to serum analysed immediately upon separation ($p$>0.05). However, there was a 0.45 mmol/L elevation in serum $Cl^-$ concentration after 5 hours compared to serum of immediately analysed after the separation. This difference was statistically significant ($p$=0.002).

**Effect of time-delay on serum ionised calcium concentration**

The $iCa^{2+}$ concentrations given by serum samples, immediately after the separation and kept for 1, 3 and 5 hours at ambient temperature are given in Table 1.

**Comparison of ionised calcium concentrations in serum**

The results showed that serum $iCa^{2+}$ concentrations were decreased with time. There was a 0.05 mmol/L reduction in $iCa^{2+}$ concentration at 3 hours compared to 1 hour and it was a statistically significant difference ($p$=0.000). The same trend was observed between 5 hours and 1 hour serum with the reduction of 0.08 mmol/L $iCa^{2+}$ in 5 hours compared to 1 hour with the $p$ = 0.000. Further, there was a 0.03 mmol/L reduction in $iCa^{2+}$ concentration at 5 hours compared to 3 hours and it was a statistically significant difference ($p$=0.021).

According to the Dunnett t (2-sided) test, there were significant differences in iCa2+ concentration in 1, 3 and 5 hours kept serum compared to the serum analysed immediately after the separation. The 1 hour serum showed a lower $iCa^{2+}$concentration compared to serum of immediately analysed after the separation and it was a statistically significantly difference ($p$=*0.004*). There was a 0.08 mmol/L reduction in $iCa^{2+}$ concentration in 3 hours kept serum compared to serum of immediately after the

separation and it was also a statistically significant difference ($p$=0.000). There was a 0.11 mmol/L reduction in $iCa^{2+}$ concentration in 5 hours kept serum compared to the serum of immediately analysed after the separation and it was statistically significant ($p$=0.000).

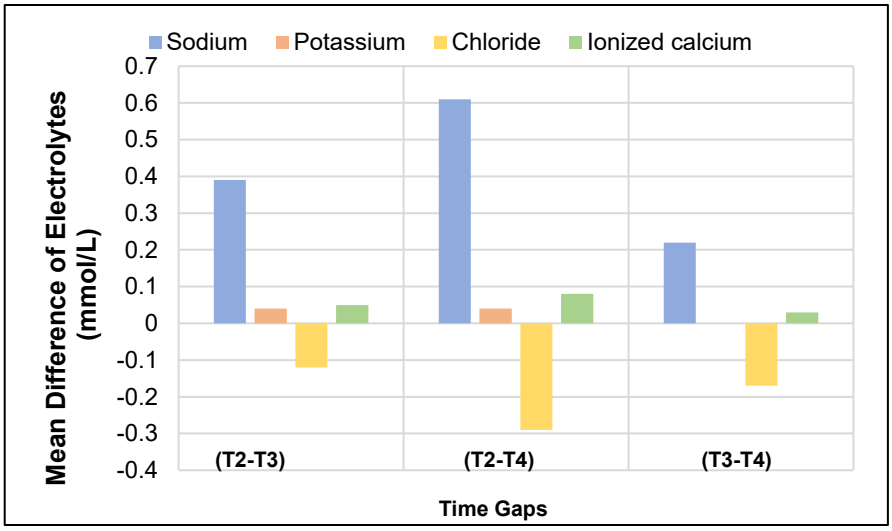



**Figure 1** Comparison of mean difference of serum $Na^+$, $K^+$, $Cl^-$ and $iCa^{2+}$ at 1, 3 and 5 hours

*Note: n=32, $T_2$ = Serum kept for 1 hour at ambient temperature, $T_3$ = Serum kept for 3 hours at ambient temperature, $T_4$ = Serum kept for 5 hours at ambient temperature*

**Mean differences in electrolyte concentrations**

Comparison of all mean difference concentrations of serum $Na^+$, $K^+$, $Cl^-$ and $iCa^{2+}$ after 1, 3 and 5 hours i.e. mean serum electrolytes differences between serum kept for 1 hour and 3 hours, 1 hour and 5 hours and 3 hours and 5 hours are shown in Figure 1 whereas comparison of electrolytes concentration in serum analysed immediately after the separation with serum kept under the ambient temperature for 1, 3 and 5 hours ie., mean serum electrolytes differences between serum analysed immediately and 1 hour, serum analysed immediately and 3 hours and serum analysed immediately and 5 hours is given in Figure 2.

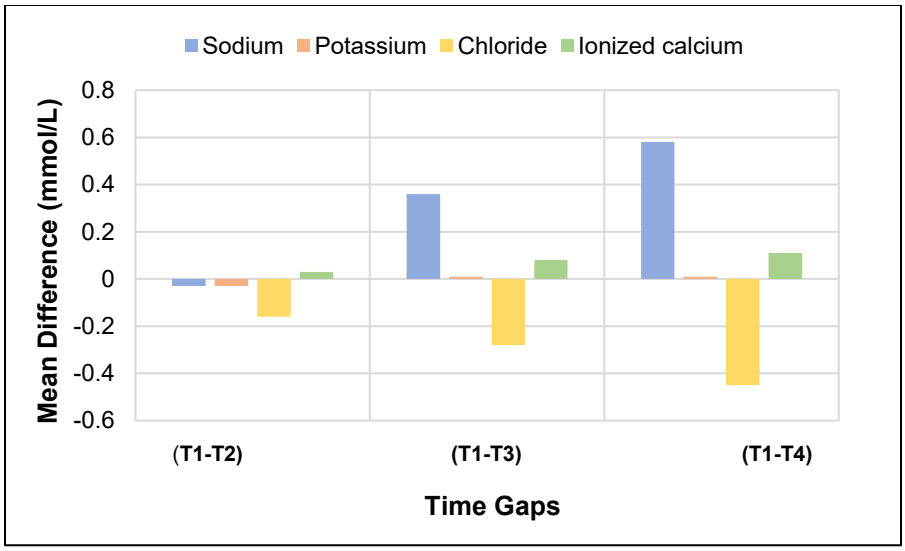



**Figure 2** Comparison of serum electrolyte concentrations at different time points with the baseline concentrations

*Note: n=32, $T_1$ = Serum immediately after the separation, $T_2$ = Serum kept for 1 hour at ambient temperature, $T_3$ = Serum kept for 3 hours at ambient temperature, $T_4$ = Serum kept for 5 hours at ambient temperature*

## Discussion

In the present study, we aimed to investigate electrolyte concentrations in plasma and serum to determine whether significant differences exist between the two sample types. Plasma separation can be performed immediately after blood collection, whereas serum separation requires a clotting period prior to centrifugation. During this delay, preanalytical changes may occur, potentially influencing electrolyte concentrations. Therefore, this study was conducted to evaluate whether the time required for serum separation contributes to significant alterations in electrolyte measurements compared with plasma.

The present study showed that stability of $Na^+$ altered after few hours of centrifugation. Serum $Na^+$ concentration decreased with time after 1, 3 and 5 hours. The study showed that significant differences of $Na^+$ concentrations between serum kept for 1 and 3 hours and 1 and 5 hours. There was also a significant difference in $Na^+$ concentration in 3 and 5 hour kept serum compared to serum analysed immediately after the separation. These findings are supported by the previous studies [8,24,25]. A pervious study showed that storing serum for a long time at 0-4 °C causes reduction of $Na^+$ concentrations. The $Na^+$ and $K^+$ in extracellular and intracellular fluid are maintained by $Na^+/K^+$ ATPase pump. ATP is required for active transportation of $Na^+$ and $K^+$ ions through cell membrane. If there is an inhibition of $Na^+/K^+$ ATPase pump, $Na^+$ is passed into the cells via concentration gradient, resulting in reduction of serum $Na^+$ concentrations [24]. Furthermore, it has been reported that serum $Na^+$ concentrations are significantly decreased in lysed blood samples as in haemolysis the serum sample is diluted as constituents of red blood cells come out. This results in reduction of serum $Na^+$ concentration

[18]. Another study showed that maximum acceptable delay for $Na^+$ in whole blood sample was 56 hours at room temperature and for serum samples for two weeks in the refrigerator [25].

In our study, baseline $K^+$ concentration in plasma and serum revealed that $K^+$ concentration in serum analysed immediately after separation is significantly higher than plasma. Further, findings of serum $K^+$ in aliquots kept at ambient temperature for 1, 3 and 5 hours showed that stability of serum $K^+$ is not altered significantly until 5 hours after the centrifugation. This finding is supported by the previous studies [24, 19]. It has been reported that $K^+$ was stable until 24 hours in both plasma and serum but $K^+$ concentration of serum was higher than plasma [20]. Razavi *et al.* (2010) showed that $K^+$ concentration in serum was higher than plasma in pediatric ICU patients [26]. Further, Hedayati *et al.* (2020) showed that maximum acceptable delay for $K^+$ in whole blood samples is 24 hours at room temperature [20]. If whole blood sample is stored too long at 0-4°C or kept more than 24 hours at room temperature led to increase in $K^+$ due to failure of $Na^+/K^+$ ATPase pump. Cool temperatures inhibit the $Na^+/K^+$ ATPase pumping action, and more $K^+$ is diffused from the erythrocytes lead to elevation of $K^+$ [24]. Another study showed that at the initial stage glycolysis is very fast, thus the serum $K^+$ concentration is decreased. When time passes, glucose in the serum is decreased and $K^+$ is passively diffused from cells and increases the concentration after long serum clot contact. This finding is in agreement with the results reported by Kalasker and Sudhamadhuri (2015) [19].

According to the present study, $Cl^-$ concentration of plasma is higher than serum analysed immediately after the separation. However, it was not significantly different ($p>0.05$). Kalasker and Sudhamadhuri (2015) reported that serum clot contact time as a major source of pre-analytical variation in serum electrolytes [19]. Further, Hedayati *et al.* (2020) showed that maximum acceptable delay for $Cl^-$ in whole blood and plasma/serum is 24 hours at room temperature [25].

We found that serum $iCa^{2+}$ concentration was significantly higher than plasma $iCa^{2+}$. It has been reported that glucose in the red blood cells is converted into lactic acid in glycolysis. Glycolysis is increased when the blood sample is stored at room temperature for more than 15-30 minutes, subsequently decreasing the pH of the sample. As a result, the $iCa^{2+}$ concentration is increased under the storage conditions mentioned. This observation is supported by the findings of Higgins (2020) [24, 27]. In our study, 30 minutes were taken for the serum separation, and this may be the reason for increased $iCa^{2+}$ concentration in serum compared to plasma. McDonnell *et al.* (2002) has shown that $iCa^{2+}$ concentration is decreased when the pH of serum is increased due to stronger binding of $iCa^{2+}$ with proteins in the alkaline environment [21]. It has been reported that, a longer time gap between centrifugation and sample analysis causes a significant increase in pH and a statistically significant decrease in $iCa^{2+}$ concentration. When the serum sample is exposed to the atmosphere, carbon dioxide is entered into the sample and pH is increased. This results in decrease in $iCa^{2+}$ concentration

[28]. Further, Boyanton and Blick (2002) showed that maximum acceptable delay for $Ca^{2+}$ in whole blood/plasma/serum is 24 hours at 4 °C and room temperature [20].

In addition to that, according to Baruah *et al*. (2014) falsely high serum electrolyte values could be obtained due to increased temperature up to 46 °C in summer, especially in tropical countries [22]. Moreover, the study revealed that the concentrations of $Na^+$, $K^+$ and $Cl^-$ are increased with delay in processing of the serum samples after centrifugation.

The same study showed that stability of $Na^+$, $K^+$ and $Cl^-$ was altered after few hours of centrifugation if there is a delay in analysis. The $Na^+$ and concentrations significantly increased at 3 and $K^+$ concentration increased significantly before 1 hour. A similar trend had been observed in the samples processed after 3 hours. However, the authors had been stated that all the samples had been kept with the lid opened during this period. The $Na^+$ had significantly increased at 3 and 5 hours. However, findings of this study contradict to the findings of similar studies. The possible reason for this difference may be the improper temperature maintenance in the laboratory leading to significant evaporation from the samples. Climatic conditions as well as opening sample containers under the fan for a few hours caused evaporation and falsely high serum electrolyte concentrations [22].

Donnelly *et al* (2009) showed that $Na^+$, $K^+$ and $Cl^-$ are stable for 24 hours at room temperature, 4 °C and -20 °C [23]. Rahamtalla *et al.* (2017) has shown that effects of temperature and delay in separation of serum on $K^+$ and $Na^+$ concentrations. The results of the delayed whole blood samples had been compared with the immediately separated (within ½ h) whole blood samples. The centrifuged whole blood samples had been kept at room temperature for 2 and 4 hours in this study. The $Na^+$ and $K^+$ showed a reduction during these time intervals but comparison between ½ hour and after 2 and 4 hours showed that there are no statistically significant differences in these electrolytes [29]. The present findings too did not reveal any significant difference in baseline serum $Na^+$ concentrations and serum $Na^+$ concentrations at 1-hour intervals. Balbás *et al.* (2017) had investigated the stability of plasma electrolytes in Barricor and PST II tubes under different storage conditions. Samples were analysed at six time intervals since centrifugation i.e. 0, 3, 6, 9, 12 and 15 hours. The results showed statistically significant changes in $Na^+$ and $Cl^-$ after 3 hours and in $K^+$ after 9 hours in both types of tubes at room temperature [30].

Another study showed that baseline $K^+$ concentration in serum was higher than plasma whereas the baseline $Cl^-$ concentration in serum was lower than plasma. The same study reported that significant change was not observed in serum K+ kept for 48 hours at room temperature [31]. In the present study also there was no significant difference in baseline $K^+$ concentrations and $K^+$ concentration measured after 5 hours at the ambient temperature.

In this study, plasma samples separated immediately after collection demonstrated superior stability compared with serum. This is because the clotting process that may lead to

continued cellular metabolism and analyte leakage, resulting in artificially altered concentrations. In contrast, plasma obtained using anticoagulated tubes allows rapid separation of cellular components, minimising ongoing in vitro biochemical activity and thereby preserving the true in vivo electrolyte status more effectively.

A limitation of the present study is that, although serum electrolyte concentrations may be affected by contamination from intravenous fluids, the impact of this factor could not be evaluated.

## Conclusions

Overall, plasma separated immediately after blood collection can be recommended as the sample of choice for the simultaneous determination of $Na^+$, $K^+$, $Cl^-$, and $iCa^{2+}$ in laboratory testing, as prompt plasma separation minimises preanalytical variations and helps ensure the accuracy and reliability of electrolyte measurements.

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## Ethical considerations

The study was conducted after obtaining ethical approval from the Ethical Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka (MLS/7/2022).

## Declaration of conflicting interest

All authors declare that they have no conflicts of interest.

## Authors' contributions

HFYCDF, WKGIW, and AMBP contributed to the conception of the research idea. The study was designed by AMBP and PD. Data acquisition was carried out by HFYCDF and WKGIW. Data analysis was performed by HFYCDF, WKGIW, and PD. The findings were interpreted by AMBP and PD. AMBP supervised the study and drafted the manuscript.

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