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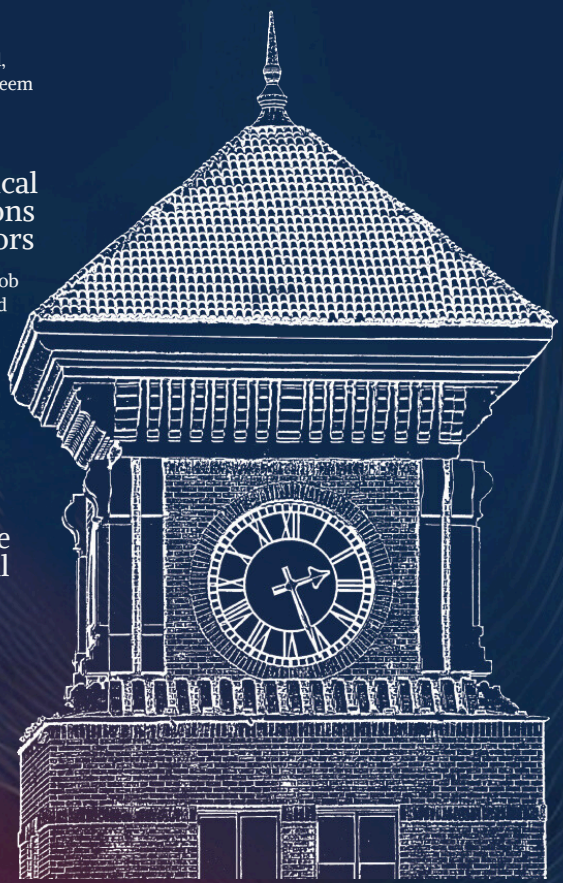

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We are pleased to present Issue 2, Volume 2 of the *UCP Journal of Science & Technology (UCP-JST)*. This milestone reflects the continued dedication and collaborative efforts of our editorial team, reviewers, and contributing authors.

We take pride in the fact that *UCP-JST* was **recognized as a Y Category journal by the Higher Education Commission (HEC) of Pakistan** in a previous issue. This recognition reflects the academic rigor and quality standards upheld by the journal. As we move forward, we remain committed to further enhancing the journal's impact and continue to strive toward achieving the next category of recognition.

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On behalf of the editorial board, I extend sincere appreciation to all contributors for their trust, scholarly commitment, and support in advancing the journal's vision.

Sincerely,
Dr. H. Rizwana Kasur
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Assessment of Blood and Renal Function Profiles in Pregnant Women from Narowal, Pakistan

Fizza Azeem[1,#], Rabia Mehmood[2,3,#], Rahat Raza Rasheed[4,#], Amna Rehman[3,#], Muhammad Zohaib[5], Suneela Amaan[6], Zoya Asif[1], Tayyba Jan[7], Hanan Afzal[2], Sadia Ahmad[3], Kaleem Maqsood[8], Ali Afzal[9], Muddasir Hassan Abbasi[4,*], Nadeem Sheikh[3,*], Muhammad Babar Khawar[1,4,*]

Abstract

Pregnancy induces significant physiological changes that can impact hematological and renal function parameters which makes them potential indicators of gestational status. The objective of this study was to evaluate whether selected haematological and renal function parameters can serve as potential indicators of pregnancy. It was hypothesized that pregnancy would significantly alter these parameters compared to non-pregnant controls. Blood samples of pregnant women and controls were analyzed for hematological and renal function parameters. Sampling was conducted in hospitals and laboratories in Narowal, Punjab, Pakistan, from February 2021 to June 2022. Blood samples from 160 pregnant women and 160 controls were collected in EDTA-coated vials for Complete Blood Count analysis. Additionally, blood samples from 51 pregnant women and 51 controls were collected in serum vials to assess renal function. A statistically significant difference was observed in haematological parameters, including haemoglobin (Hb) ($t = 4.87$, $p < 0.0001$), haematocrit (HCT) ($t = 3.92$, $p = 0.0001$), and white blood cells (WBC) ($t = 2.76$, $p = 0.0063$). Renal function analysis revealed distinctive differences, with blood urea showing a significant increase in pregnant women

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(mean ± SD: 34.6 ± 8.2 mg/dL) compared to controls (28.1 ± 6.7 mg/dL), t(100) = 3.76, p = 0.0002, and serum creatinine levels also significantly higher in the pregnant group (0.89 ± 0.15 mg/dL) versus controls (0.76 ± 0.12 mg/dL), t(100) = 3.70, p = 0.0003. These findings suggest that specific hematological and renal function tests may serve as descriptive indicators of physiological changes during pregnancy. Further studies on larger populations are recommended to validate these results and provide more precise information.

Keywords: Pregnancy; Complete Blood Count; Hematology; Renal Functioning Test; Biomarker

1. Introduction

Pregnancy, also known as gravidity or gestation, is the period during which a woman's womb creates one or more babies (Chandra et al., 2012). Pregnancy is a period in which the fetus survives and grows in the body of the mother and the immune system of the mother body does not reject the implantation of a fetus (Mazza, 2007). This gestation period is alienated into 3 trimesters. The first trimester is of less than 14 complete weeks, the second trimester is 14 to 27 complete weeks, and the third trimester is of 28 complete weeks till delivery (Hurjui et al., 2017).

Certain physiological changes affect the alimentary canal, cardiovascular, pulmonary, and renal organs to provide nutrients and remove waste (Kaur et al., 2014). The fetus's blood volume increases to oxygenate vital organs (Osonuga et al., 2011), leading to hyperpigmentation specially in darker areas (Ahmed et al., 1993). Maternal blood pressure drops during the first and second trimesters, returning to baseline in the third trimester (Khellaf et al., 2012). During pregnancy, sodium retention increases due to factors like estrogens, cortisol, renin-angiotensin, aldosterone, and posture. Pregnancy affects potassium balance and excretion, with a less precise regulation mechanism than sodium (de Flamingh & van der Merwe, 1984). Estrogens and progesterone moderate potassium excretion, enhancing sodium reabsorption and acting as mineralocorticoid antagonists (Lindheimer et al., 1987). Lactate dehydrogenase (LDH) levels may rise from pre-pregnancy levels in the first trimester to normal levels in the third trimester. Other authors claim that LDH has remained unchanged (Cattozzo et al., 2013). During the first trimester, significant variation in renal plasma flow and glomerular filtration rate were already reported (Teasdale & Morton, 2018).

There are many physiological, hematological and serological parameters that significantly fluctuate during pregnancy, which are otherwise an indication of diseased condition in non-pregnant women (Anwar et al., 2023; Chandra et al., 2012). Hematological parameters have been increasingly studied as disease-specific biomarkers across a variety of physiological and pathological conditions. Our previous investigations have demonstrated significant alterations in CBC profiles in patients with infectious diseases (Rasheed et al., 2022), inflammatory skin conditions (Nawaz et al., 2022), and renal pathologies (Butt et al., 2023). This suggests that the broad diagnostic potential of blood-based markers. Therefore, we aim to explore whether fluctuations in blood profiles and renal function tests may indicate pregnancy-related physiological changes. Pregnancy is related to hematological and renal alterations, such as changes in hemoglobin, white blood cells, and serum creatinine levels. While these parameters appeal promising biomarkers, further

validation in larger and more diverse populations is necessary.

2. Methodology

2.1. Subjects

Our case-control investigation involved 160 pregnant women and 160 healthy non-pregnant women as controls. An additional 102 blood samples were collected for renal function test (RFT) screening, comprising 51 samples from pregnant women and 51 from healthy controls. All participants provided written informed consent before enrollment. To minimize potential bias, all subjects were recruited exclusively from the same geographical region, specifically various cities in District Narowal, Pakistan. The study was approved by the Ethical Committee of the University of Narowal, Narowal, Pakistan.

2.2. Inclusion and exclusion criteria

Inclusion criteria for pregnant participants were: confirmed pregnancy by ultrasound, age between 18–40 years, and no known pre-existing chronic illnesses. Controls were age-matched, non-pregnant women with no history of chronic disease. Exclusion criteria for both groups included any known renal disease, hematological disorders, recent infections, or current use of medications affecting renal function or hematological parameters.

2.3. Sampling and Hematological Analyses

Three milliliters of venous blood were collected aseptically in EDTA-coated vials from all participants. Complete blood count (CBC) was performed on these samples using an automated hematology analyzer (XP-300, Sysmex Corporation, Japan). The analyzer was calibrated daily according to the instructions, and validation was performed using commercially available quality control materials to ensure accuracy and precision, as previously described (Butt et al., 2023).

2.4. Measurement of Serum Urea and Creatinine

Under aseptic conditions, hemolysis-free serum was separated. Ready-to-use kits (e.g., Pfizer) were used for estimation of serum urea and creatinine following the manufacturer's guidelines. All assays were performed in duplicate to confirm reproducibility. The analytical methods employed in this study were consistent with protocols established in our previous investigations involving CBC and renal function testing in clinical populations across Punjab (Afzal et al., 2024; Rasheed et al., 2022; Riasat et al., 2022).

2.5. Statistical Analysis

Data were analyzed using Prism GraphPad version 8. An unpaired t-test was applied to assess statistically significant differences between groups. A p-value less than 0.05 was considered statistically significant. In addition to p-values, 95% confidence intervals were calculated to evaluate the precision of estimated differences, and effect sizes (Cohen's d) were reported to indicate the magnitude of the observed effects.

3. Results

Investigation of complete blood count leads to the finding of certain significant changes. A statistically significant decrease was found in the levels of hemoglobin (Hb) and hematocrit (HCT) of pregnant women when compared with the non-pregnant women. Comparison by t test revealed a marked increase in count of white blood cells (WBCs) during pregnancy with a level of significance $p<0.0001$ (Figure 1).

The results implies that the renal function test of the control and pregnant women samples showed a distinctive difference with the high variance in serum urea and creatinine. The levels of both markers i.e., serum urea and serum creatinine decreased significantly in pregnant women with a level of

significance p=0.0002 and p=0.0003 respectively (Figure 2).

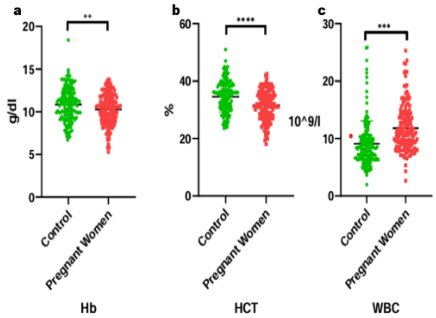

Figure 1 Hematological parameters (Hb, HCT, WBC) in pregnant women (n=160) vs controls (n=160). Data are presented in mean ± S.E.M.; *P≤0.05, **P≤0.01, ***P≤0.001 (unpaired t-test).

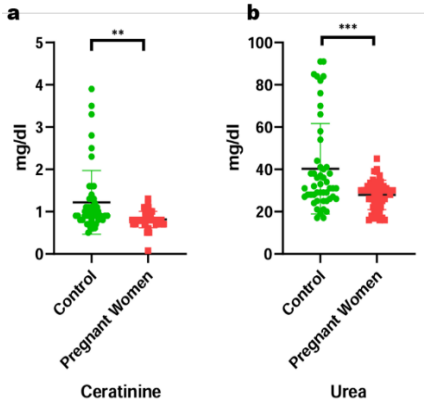

Figure 2 Serum creatinine and urea levels in pregnant women (n=51) and controls (n=51). Mean ± S.E.M.; *P<0.05, **P<0.01, ***P<0.001 indicate statistical significance.

4. Discussion

We observed notable differences in CBC parameters and RFTs between pregnant and non-pregnant women. Our results showed that hemoglobin and hematocrit levels were lower, while white blood cell counts were elevated during pregnancy. These findings align with previous reports indicating that pregnancy-related anemia is among the most prevalent hematologic issues faced by clinicians. The reduction in hemoglobin is likely due to increased iron requirements to support fetal growth and maternal blood volume expansion, which are often unmet by dietary intake alone (Li et al., 2017). The current findings showing significant changes in hemoglobin, hematocrit, and WBC counts during pregnancy are consistent with our earlier work where similar CBC parameters were found to differentiate patients with hepatitis B and psoriasis from healthy individuals (Nawaz et al., 2022; Rasheed et al., 2022). The observed elevation in blood urea and serum creatinine among pregnant participants is aligned with previous findings from our lab, where such renal function markers potently distinguish hemodialysis patients from healthy controls (Riasat et al., 2022).

The observed leukocytosis is consistent with established evidence showing a physiological rise in total WBC counts during pregnancy. Studies have demonstrated that neutrophil counts, in particular, increase steadily between 8 and 40 weeks of gestation, reflecting a response to physiological stress and hormonal changes (Chandra et al., 2012) (Dockree et al., 2021). Leukocytosis, a significant change in hematological profile during pregnancy, is caused by physiological stress and increases in white blood cell (WBC) count (Lurie et al., 2008). Moreover, leukocytosis has been reported to persist postpartum before gradually returning to pre-pregnancy levels over several weeks (Wadsworth, 2002) (Kaur et al., 2014). Our findings further support the concept that elevated neutrophil counts are a normal adaptive response during pregnancy, with counts potentially reaching up to twice those observed after delivery (Paidas & Hossain, 2010).

Regarding renal function, we found that serum urea and creatinine levels were significantly lower in pregnant women compared to controls. This is in agreement with earlier studies reporting decreased creatinine concentrations as a normal physiological change during pregnancy due to increased glomerular filtration rate (Girling, 2000). However, it is important

to interpret these reductions cautiously, as elevated levels can indicate underlying pathology. For instance, higher serum creatinine and blood urea nitrogen levels have been documented in pregnant women with pregnancy-induced hypertension (Tewabe & Wolde, 2020), while creatinine concentrations above 77 μmol/l (0.87 mg/dl) may suggest acute kidney injury or previously unrecognized chronic kidney disease (Wiles et al., 2019). Lower urea and creatinine levels likely reflect increased glomerular filtration and renal clearance during pregnancy. These physiological adaptations help manage metabolic demands of the developing fetus.

Current study builds upon a body of work conducted by our research group, which has consistently shown that CBC and serum biomarkers offer valuable insight into physiological stress and systemic changes associated with various clinical conditions (Afzal et al., 2024; Butt et al., 2023; Nawaz et al., 2022; Rasheed et al., 2022; Riasat et al., 2022). Specifically, our earlier study on kidney stone patients emphasized how hematological profiles, including neutrophil-to-lymphocyte ratio (NLR), can serve as early indicators of renal complications (Butt et al., 2023), reinforcing the current study's use of such parameters to evaluate pregnancy-related renal shifts. Moreover, the use of CBC markers to detect immune or inflammatory imbalance, as in psoriasis and hepatitis B patients (Nawaz et al., 2022; Rasheed et al., 2022), supports the present analysis of immune-related hematological changes in pregnancy. These collective findings contribute to our ongoing effort to identify low-cost, non-invasive markers that may be useful for early screening or monitoring of various conditions in resource-limited settings. Although current study provides insightful results, there are certain limitations. Our study was limited by its relatively small sample size for renal function analysis, which may affect the generalizability of the findings. Additionally, potential confounding factors such as dietary intake, hydration status, and underlying subclinical conditions were not controlled, which could influence hematological and renal parameters during pregnancy.

5. Conclusion

Our investigation gives proof of the differentiation between pregnant and normal women because of the CBC and renal profile results. The results of the CBC and renal functioning profile trial of normal women were altogether not quite the same as cases of pregnant women. As convenient biomarkers, hemoglobin, hematocrit, white blood cell count, serum urea, and serum creatinine can serve as useful indicators for evaluating physiological changes in pregnant women. These findings are important for future research and their potential application in clinical practice.

6. Declarations

6.1. Conflict of interest

The authors declare no potential conflicts of interest.

6.2. Acknowledgments

The authors would like to acknowledge the Biorender that aided in the creation of figure.

6.3. Funding statement

No funding was received for this study.

6.4. AI Tool Declaration

During the preparation of this work the authors used ChatGPT in order to simplify the content. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Water Chestnut (*Trapa natans*): Physicochemical Properties of Starch and Industrial Applications across Food, Textile and Pharmaceutical Sectors

Muhammad Irfan[1*], Mubeen Sultan Butt[2], Muhammad Bilal Haider[3], Mehboob Ahmad[4], Talha[4], Bisma Khaliq[1], Muhammad Mubashir Ramzan[1], Muhammad Muzamil[1], Sana Aslam[4]

Abstract:

Water chestnut (*Trapa natans*) are full of nutrition, which has drawn the attention of researchers because of their exceptional starch attributes and diverse applications. They are known for their high carbohydrate; dietary and essential minerals content, as well as low caloric profile. Water chestnut take part notably in a balanced diet and are incorporated into different culinary applications globally. The starch from *Trapa natans* shows considerable physicochemical properties, including gelatinization temperature, resistance to retrogradation and superior water-holding capacity, making it a promising constituent for food and non-food industries. In the food sector, water chestnut is used as a thickening agent, stabilizer and texturized in gluten-free products, while its bioactive compounds find applications in functional foods. Beyond the food industry, this starch has potential in the textile and pharmaceutical sectors, providing environment-friendly alternatives for fabric finishing, biodegradable packaging films and excipients in drug formulations. This review provides a comprehensive overview of the nutritional properties of water chestnuts, their physicochemical properties and diverse industrial applications exhibiting their capacity to accelerate product innovation across multiple sectors.

Keywords: Water chestnuts, *Trapa natans*, Starch physiochemical properties, Industrial applications

1. Introduction

Trapa natans L., commonly known as "water chestnut" or "singhara nut," belongs to the family Trapaceae and is referred to as "singhara" or "simkhata" in Hindi, and "karimbolam" or "vankotta-kkaya" in Malayalam. Despite its high nutritional value, water chestnut is underutilized in food processing due to its seasonal availability of only two to three months each year. Globally, it is cultivated on approximately 603,076 acres, yielding a total of 2,327,495 tonnes. The fruits of *Trapa* are known for

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their cooling, diuretic, sweet, astringent, and tonic properties (Patel et al., 2011). Despite their name, water chestnut are not actually nuts. Instead, they are aquatic tuber vegetables that thrive in shallow water bodies, such as lakes, paddy fields, marshes, and ponds. Native to several islands across the Indian and Pacific Oceans, water chestnut are also widely found in regions of Southeast Asia, southern China, Taiwan, Australia, and parts of Africa (Rajput & Singh, 2023).

It is harvested when deep brown, water chestnut have crisp, white flesh enjoyed raw or in Asian dishes like stir-fries and curries (Rajput & Singh, 2023). Water chestnut are mainly regarded as a vegetable and are commonly used in human foods, particularly in sweet dishes and baked goods, often in the form of dried flour (Patel et al., 2010). Raw water chestnut are 74% water, making them a low-calorie option and a good source of fiber, potassium, copper, manganese, vitamin B6, and riboflavin (Rajput & Singh, 2023).

During freeze-thaw cycles, water chestnut starches exhibited greater syneresis and reduced paste clarity over storage time. Some sources demonstrated enhanced swelling power, water-binding capacity, freeze-thaw stability, solubility and viscosity. However, these sources had lower protein levels, pasting temperature, amylose content and gel firmness (Gani et al., 2010). Starch serves as a thickening, bulking, and gelling, stabilizing, and water-retaining agent, widely used in food processing. Both native and modified starches enhance the texture and quality of products, with modified starches now regarded as essential functional food additives (Gani et al., 2010). Water chestnut possess low content of fat, ash and protein and higher carbohydrate content, which make it more efficient and its extraction is starch cost-effective than the starch from other sources. The lowest impurity in water chestnut starch make it a best candidate to be used as a pharmaceutical excipient, used for controlled release-formulations, such as tablets (Kaur et al., 2023). Moreover, its gelling, binding and thickening characteristics prove it to be a useful candidate in paper, biomedical, textile and other commercial applications (Syed et al., 2021). Water chestnut starch stands out for its unique ability to develop a firm, crunchy texture when heated, unlike conventional starches like potato or rice. This property makes it highly beneficial for the food and other related industries. A complete examination of its characteristics could accelerate its industrial applications, with the capacity for exact physicochemical modifications to fulfill particular requirements (Gani et al., 2010). Reviewing the literature, it is acknowledged that water chestnut starch has excellent nutritional profile and wide range of applications across the pharmaceutical, textile and food sectors as indicated in Figure 1:

Figure 1 Applications of water chestnut starch

2. Chemical Composition

Water chestnut provide important minerals, lipids, proteins, carbohydrates and variety of vitamins (A, B, C A, and E). Dietary fibers and polyphenols, including flavonoids, phenolic acids and hydrolysable tannins are also present in it (Patel et al., 2010). A significant amount of phenolic acids, including gallic, ellagic and ferulic acids are present (Aleksic et

al., 2018). The primary active compounds in the aquatic fruit extract of water chestnut are phenolics, which have attracted scientific interest due to their potent anti-free radical properties. The high polyphenol content in water chestnut fruit aqueous extract is supported by evidence demonstrating significant in vitro antioxidant activity against free radicals (Corovic et al., 2021).

Table 1 Chemical composition of water chestnut

Component	Percent
Water	80.9
Lipids	0.37
Total ash	1.32
Crude fibre	0.71
Total proteins	1.86
Amylose	29

(Singh et al., 2017; Kaur et al., 2023)

3. Food Applications

Water chestnut fruits can be dried and ground into flour, which is sometimes used as a substitute for arrowroot flour. They are also enjoyed roasted or boiled. With a nutritional profile that includes 16% carbohydrates and 2% protein, these fruits offer substantial nourishment. Raw water chestnut are juicy and crisp, while cooking softens them but keeps their crunchy texture. Water chestnut kernels are highly nutritious, containing a rich array of vitamins, carbohydrates, minerals and essential elements such as iron, manganese, copper, magnesium, sodium, and potassium (Rajput & Singh, 2023). At Wular Lake in India, approximately 4-5 million kilograms of water chestnuts are harvested each year, providing sustenance for nearly 30,000 people over a five-month period. In nations like Bangladesh, China, and India, water chestnuts are widely sold by street vendors, either fresh or cooked. The younger pulp, known as "milky" water chestnut, is enjoyed both raw and cooked, while the mature pulp is typically boiled, dried, and incorporated into various recipes. Besides being consumed as a vegetable, water chestnuts are also used to prepare tea, curries, and flour for breads like chapattis and poories, and are available in dried form as nuts. Nutritionally, water chestnuts are comparable to wheat (Malviya et al., 2010). To cure high blood pressure in pregnant women, the porridge prepared with water chestnut flour is recommended as water chestnut flour accelerates fatal growth. The women having bleeding issue, can utilize dried-seeds to overcome the issue. Moreover, its juice exert several therapeutic uses, including the elimination of bile (Rajput & Singh, 2023). Boiled water chestnut is a common form for consumption, and in India, it is valued for its high nutritional content, making it suitable for premium chapattis and sweets. Increasingly, people across socioeconomic backgrounds globally use this medium-to-low calorie food for snacks due to its rich nutrient profile In developing countries like India, malnutrition remains a recognized issue, which experts have aimed to address through scientific research and novel food formulations derived from various nutritional sources to enhance diets (Sachin Parmar, Amit Gangwal, 2011).

Non-traditional food sources have gained significant interest for their potential as functional ingredients in new food products. The starch characteristics of water chestnut (*Trapa natans*) are shaped by its physicochemical properties, such as mineral composition, growth period, variety, grain size, environmental conditions, and the amylose-to-amylopectin ratio (Mahnoor et al., 2024). It also helps improve food product shelf life and stabilize gels during processing. India's bakery industry, one of the country's largest food sectors, ranks as the world's second-largest biscuit producer after the USA. Given the high prevalence of celiac disease, especially in North India, there is a growing demand for

gluten-free products, as these are essential for managing the condition and improving the quality of life for affected individuals. Consequently, gluten-free options are expanding globally in response to the rising celiac diagnosis rates (Kaur et al., 2023)

Water chestnut (*Trapa natans*) is widely used in the Indian subcontinent as a substitute for cereals during fasting and is considered a suitable replacement for wheat flour (WF), especially beneficial for individuals with Celiac disease due to its gluten-free nature (Mir et al., 2015)**.** Additionally, the flour obtained from water chestnut can be added into several food products like noodles and cookies (Choudhury and Chaudhary, 2023). In the same way, mixing wheat and water chestnut flour contributed to the shortening of cooking time of noodles. Moreover, the mixing resulted in the alteration of texture profile and retaining of overall desirability of noodles developed with composite flour than that of traditional wheat flour. These results suggested the huge potential of water chestnut flour to be a potential candidate in the development of novel foods (Hussain et al., 2019). In India, cookies made from water chestnut flour are a popular delicacy during Navratri and other fasting days. Its high fiber content and various nutritional benefits further support its use in food applications. Compared to WF cookies, water chestnut flour cookies show a higher spreading ratio, likely due to WCF's elevated starch content, which has fueled demand for this alternative flour (Singh et al., 2011). Research on modified water chestnut starch revealed its positive influence on sponge cake characteristics; the addition of acetylated starch significantly improved the volume index, while acid-thinned starch at a concentration of 1% w/w enhanced the symmetry index. At a rate of 4–5% w/w, pre-gelatinized and acid-thinned starches achieved a notable uniformity index. In a separate study, yogurt enriched with water chestnut starch was evaluated in comparison to yogurt containing 0.5% w/w gelatin as a stabilizer (Lutfi & Hasnain, 2009)**.** Water chestnut starch improved yogurt's syneresis, water-holding capacity, and viscosity, achieving high sensory acceptance without affecting taste or quality. Optimal results for water-holding capacity, syneresis, and viscosity were observed with water chestnut starch at 1.25% w/w and 0.75% w/w, extending yogurt's shelf-life up to four weeks (Hallale & Jadhao, 2016).

Starches, especially from water chestnut, are crucial in food and industrial applications due to their thickening properties, and are often used to improve texture in foods like sauces and soups. Water chestnut starch has an amylose content of 22.3% w/w, closely linked with amylopectin, although it's generally not applied as a stabilizer in dairy products (Hallale & Jadhao, 2016)**.** However, research indicates that water chestnut starch purified from Trapa bispinosa Roxb contains 7–8% moisture, 0.21–0.22% protein, 0.03–0.04% ash, and 81–85% carbohydrates, delivering 385 calories per 100g. When used in dahi at varied concentrations, water chestnut starch significantly affected dahi's physical, chemical, and sensory qualities, showing excellent stability by reducing syneresis and enhancing creaminess and texture without impacting flavor or quality. Studies further indicate that dahi with added water chestnut starch remained viable for up to one month under cold storage. Additionally, water chestnut starch, both native and modified, serves as an effective fat substitute in mayonnaise, potentially lowering its calorie count. Mayonnaise made with succinylated water chestnut starch showed superior sensory and textural

quality over acetylated starch formulations (Ansari et al., 2017).

Biodegradable and edible films are plant-based packaging materials, which are increasingly used to extend the shelf life of food and reduce environmental problems. These materials are being consumed along with the food, which enhance nutritional and sensory attributes of food. Antimicrobial agents are also added to edible films to prevent microbial contaminations on food surfaces (Basch et al., 2013). In this context, water chestnut is exhibiting as a sustainable packaging material for food and non-food uses (Singh et al., 2009). The antimicrobial properties of films prepared with water chestnut starch-chitosan were accelerated with the addition of some additives, including nisin, glycerol monolaurate, *calendula officinalis* extract and pine needle essential oil (Mei et al., 2013). These additives effectively inhibited the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* across different concentrations. Another study developed a biodegradable film from water chestnut starch and polyvinyl alcohol (PVA), observing that plasticizer additions decreased the swelling power and increased solubility of the films, with lower plasticizer concentrations enhancing tensile strength (Zehra et al., 2022). Additionally, clusteroluminogenic films made from maize, potato, and water chestnut starch revealed that water chestnut films had the highest transmittance. These films maintained consistent clusteroluminescence in fresh and frozen food but showed decreased luminescence after thawing, suggesting potential use as an indicator of storage conditions for frozen foods (Lai & Wong, 2022).

It was investigated the effects of edible coatings made from water chestnut powder on the quality and shelf life of Kala Kullu apples. They tested two coating concentrations: T1 (2% water chestnut powder) and T2 (2.5% water chestnut powder), using 2% guar gum as a positive control and leaving some apples uncoated as a negative control (Mahnoor et al., 2024).

3.1. Physicochemical Properties

The swelling capacity of starch reflects the degree of interaction between its crystalline and amorphous regions. This capability is primarily determined by the granules' water retention, facilitated by hydrogen bonding. During gelatinization, hydrogen bonds that stabilize the double-helix structure of starch crystallites break, allowing new hydrogen bonds to form between exposed OH groups in amylose, amylopectin, and water molecules. Partially decomposed starch granules absorb water more conveniently as compared to whole ones, which enhances their potential to swell. Granule's disintegration and the composition of starch considerably affect swelling along with gelatinization temperatures. Water chestnut starch is recognized for its high swelling capacity, likely attributed to its lower amylose content and a higher proportion of impaired starch granules (Kaur et al., 2023). It was observed this trait within a temperature range of 40 °C to 95 °C, with measurements taken at 5 °C intervals. Notably, swelling ability surged sharply at 50 °C, then continued to rise moderately with increasing temperature until reaching the gelatinization point (Cai et al., 2014). It was found that both annealing and heat moisture treatment reduce starch granule swelling power and solubility, suggesting strong forces within the crystallite regions of modified starches. During annealing, amylose chains shift to helical structures, increasing inter-chain attractions and lowering swelling power. Increased moisture content also enhances interactions in crystalline regions, limiting

Table 2 Comparison analysis of characteristics of water chestnuts, potato and maize starches

Characteristics	Water chestnut starch	Potato starch	Maize starch
Amylose (%)	21.8-32.10	26.8-30	25-28
Swelling (g/g at 90 °C)	1.4-11	51.2	10.9
Solubility (% at 90 °C)	17	13.87	18.7
Water absorption capacity (%)	133.4	125-140	115-130
Gelatinization temperature(°C)	69.60	59.9	68.7

(Kaur at al., 2023; Song et al., 2013; Jansky & Fajardo, 2016; Obadi et al; 2023, Yousif et al., 2012)

swelling. Heating starch suspensions causes granule hydration, swelling, and some solubility. When measured in water chestnut starch, swelling varied by lake source, with Anchar Lake starch showing 1.4% to 11% w/w swelling and Wular Lake 2.2% to 15% w/w, while Dal Lake starch peaked at 4.2–18.3% w/w. Dal Lake samples also showed higher solubility (3.4–17% w/w) compared to other sources. Swelling was limited below 70 °C but increased between 70 °C and 90 °C due to hydrogen bond disruption in amorphous regions (Gani et al., 2010).

Higher amylose content enhances interactions with amylopectin chains, resulting in increased gel shrinkage and syneresis. The syneresis of starch gels can be decreased by increasing the level of acids (Pulgarín et al., 2023). The reduction in starch syneresis during the formation of gel can be reduced by lower amylose. This is due to the low water uptake by starch granules during their breakage and stiffening effect that heat and moisture treatment applies on the granules (Singh et al., 2019). On the other hand, heat and moisture treatment have tendency to increase syneresis than native starch. The increase in the light transmittance of water chestnut starch was observed with the addition of salt, particularly at the concentrations of 0.5% and 1% by w/w. The breakdown of hydrogen bonds within the starch structure and between starch-water molecules improves the transmittance of light. This improvement in transmittance resists the rearrangement of starch during retrogradation. That is why salt-treated starch gels give higher transmittance than untreated native starch gels. Furthermore, retrogradation is significantly reduced by treating water chestnut starch with extrusion polyphenol, which boosts transmittance levels (Lutfi et al., 2019).

The changes in phase are observed from an organized to disordered form when the mixture of starch and water is applied with shear heating at high temperatures of gelatinization. The change in arrangement enhances the viscosity of the mixture, which leads to the formation of starch paste. The length and amount of amylose chains and branching and the size of amylopectin chains are the factors which influence the starch paste (Reddy et al., 2017; Cruz et al., 2013). The physiochemical and morphological properties are changed by repeated freeze-thaw cycles in starch containing foods, including soups, sauces and ice creams. In the start, a consistent starch gel is formed upon gelatinization, but gel becomes disordered due to freezing. Continuous freeze-thaw cycles change into cryotropic gels, conclusively offering the finished products a spongy texture (Gong et al., 2024).

3.2 Comparative analysis of starch characteristics from different sources

The starch originated from water chestnut possess small; uniformity and granular structure as compared with potato starch having exceptional shapes while same in size. It has higher high amylose concentration, which exhibits capacity for the development of resistant starch and digestibility (Dularia et al., 2019). It has considerably significant swelling potential with medium release of water when thawed that exhibits its functionality with some improvement gaps (Alam et al., 2021). A brief comparison of physicochemical properties of starch from different sources is presented in Table 2.

4. Industrial Applications

Starch is a naturally originated glucose homo-polysaccharide of nutritional and industrial significance. The complexity of structure and poor solubility of starch (native) in water reduces its industrial use. The functionality, structure and reactivity of the native structure can be subjected to modification by various methods, including chemical, physical and enzymatic (Nawaz et al., 2020). The water chestnut starch can be modified and processed into various commercial products as shown in Figure 2:

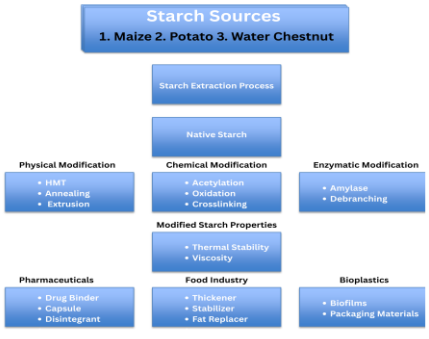

Figure 2 Modification of starch for industrial applications

Starch is one of most searched pharmaceutical ingredients worldwide, famous for its bio-stability, availability, and diverse attributes. The starch collected from maize and potato is used in tablet formation as a binding agent, while research is being carried out to explore alternative botanical sources (Okunlola & Odeku, 2011). Binders have a great role in formulating tablets, which provides crucial adhesion between granular ingredients to make exceptional bonding during compression. Plasticity is increased by the incorporation of binders, which accelerates the rate of compaction and improves the bonding between particles. Furthermore, the risk of brittle fracture during the tablet formulation process by Additionally, the risk of brittle fracture is decreased by the addition of binders (Khairnar et al., 2024). A few industrial applications are shown in Table 3:

Table 3 Application of water chestnut in industrial sector

Industry	Application	Property
Pharmaceutical	Tablet	Binder
Pharmaceutical	Encapsulation	Coating
Plastic	Bioplastics	Binder
Nutraceutical	Nano-capsulation	Coating
Textile	Removal of rhodamine B	Bio-adsorbent

(Rao et al., 2011; Ahmad et al., 2019; Kulkarni & Badwe, 2020; Khan et al., 2013)

5. Processing Limitations and Mitigation Strategies

The processing of water chest of nuts faces various constraints, especially because their high moisture content and sensitivity to spoilage. Water chestnut obtained from freshwater are more susceptible to microbial contamination, which leads to quick deterioration that faces challenges for transport and storage. They need additional processing steps due

to their fibrous skin, which is labour intensive and increases processing costs. Furthermore, water chestnut starch is very sensitive to pH and temperature, which can reduce its functionality during processing at industrial scale. Preservation techniques and drying methods are usually applied to retain the quality of the product, however these can be energy-requiring and cost demanding. Resolving these challenges is necessary to enhance the efficiency and cost-effectiveness of water chestnut processing at industrial scale.

Starch extracted from water chestnut have tendency to undergo retrogradation or decomposition when subjected to heat and pH changes. Carboxymethylation, succinylation and acetylation, collectively known as CMS, enhances swelling, paste clarity, resistant start content and solubility, which make it an ideal candidate for food and pharmaceutical industries (Xiao et al., 2018). To improve the stability of water chestnut starch, the pasting temperature is accelerated and viscosity breakdown is reduced during hot processing. Moreover, Packaging films containing water chestnut starch nanoparticles enhanced tensile strength, limited the solubility and transmission through water vapor, which make it more efficient (Dularia et al., 2019). Application of dry heat with alginate can mitigate solubility and swelling but enhanced water absorption and thermal response, which support the controlled release (Lutfi et al., 2021).

6. Conclusion

In conclusion, water chestnuts have notable significance due to their nutritional profile and diverse applications in the food, pharmaceutical and textile industries. Despite this potential, the processing of water chestnuts has considerable limitations; high perishability and microbial spoilage remain basic challenges, often requiring special storage and preservation methods to extend their shelf life. The original form of starch that originated from water chestnut is susceptible to temperature and pH, which obstacles its utilization in various thermal applications. Chemical or physical alterations are mostly crucial to boost solubility, textural and thermal characteristics for industrial applications. However, it dominates over many for its various physiochemical properties. Its cultivation is usually seasonal and regional, which may have an effect on constant supply for commercial applications. Nevertheless, its growth ability in marginal lands makes it a sustainable option. More research studies are required to assess consumer acceptability. Moreover, the peeling process is laborious work which raises its processing costs and limits its usage on a large scale. Additionally, a lack of standardized processing methods for water chestnut-based products cannot maintain consistency and quality in end-products. Addressing these limitations through technological innovations and optimized processing techniques could accelerate the market potential of water chestnuts, making way for the sustainable rise in different sectors.

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Efficient Cytomegalovirus Detection Among Haemodialysis Patients Through qPCR

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Abstract

One of the leading causes of death in Hemodialysis (HD) patients is Cytomegalovirus (CMV) infection. Quantitative PCR is considered as a highly sensitive method as it detects small quantities of nucleic acid in various clinical samples. In Pakistan, nephrology units are not well developed and the investigations involved in normal encounter of viral infections in HD patients have been quite limited. Present study aimed to determine the prevalence of CMV infection, by ELISA and qPCR in patients undergoing HD, in nephrology units in Pakistan. In this cross-sectional study total 150 patients undergoing HD were included by non-probability convenience sampling technique, and those undergoing peritoneal dialysis were excluded. Blood specimen was collected to estimate Hb, TLC, PLT count, bilirubin total, AST, ALT, ALP, urea, creatinine, uric acid and glucose levels. CMV IgM and IgG antibodies were detected by ELISA method and CMV viral load by qPCR. Data was analyzed by using SPSS v.26. Mean of age was found to be 42.28±13.63 years, CMV IgM antibodies level=323±210 AU/mL and CMV IgG antibodies level=311±182 AU/mL. Overall patients' frequency of male was 66.7% and of female was 33.3%. Frequency of patients with positive CMV IgM level was 43.3%, positive CMV IgG level 72.0%, and positive for CMV viral load 82.7%. Prevalence of CMV IgM infection was higher in younger age (45.1% vs. 40.7%), males (45.0% vs. 40.0%), anemic males (46.0% vs. 38.5%), leukocytosis (55.6% vs. 50.0%), thrombocytopenia (32.0% vs. 18.2%). Prevalence of CMV IgG infection was also higher in younger age (74.7% vs. 67.8%), anemic males (73.6% vs. 61.5%), anemic females (73.2% vs. 66.7%), and leukocytosis (80.0% vs. 75.0%). Prevalence of CMV viral load was lower in younger age (81.3% vs. 84.7%, P-value 0.748); males (81.0% vs. 86.0%, P-value 0.593); non-anemic males (76.9% vs. 81.6%, P-value 0.708), leukocytosis (93.3% vs. 100.0%, P-value 0.019), bilirubin total >1.0 (80.4% vs. 83.8%, and BSR (78.7% vs. 88.5%, P-value 0.177). The study concluded that prevalence of positive CMV viral load was markedly higher than prevalence of CMV IgG positive cases and almost twice higher than CMV IgM positive cases. Screening for CMV infection by commonly used ELISA method may give false negative results. Therefore, patients undergoing HD should be screened by quantitative PCR in future.

Keywords: Cytomegalovirus, Haemodialysis, ELISA, qPCR, IgG Antibodies, IgM Antibodies, Leukocytosis, Bilirubin, ESRD, BSR.

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1. Introduction

Cytomegalovirus (CMV) is highly prevalent worldwide, among all socio-economic groups and infects 50.0% to 85.0% of adults of age near 40 years. Its infections are usually endemic in the community and are mostly asymptomatic in childhood. In Africa and Latin America, seroprevalence of CMV ranges from 40-60% and 80-100%, respectively, while in Brazil, ranges from 65-85% (Fowler et al., 2022). The total rate of congenital HCMV infection is proportionate to the population's seroprevalence. Latency of CMV is more widely spread nowadays (Schwartz & Stern-Ginossar, 2023). There were more than 4,000 cases in the UK/year in 1972. Good hygiene practices and reduced close contact between adults & children have decreased the prevalence rate in most of the advanced countries (Jones et al., 2023).

CMV is a double stranded DNA human Herpesvirus, which belongs to the subfamily 'beta-herpesvirinae' of family Herpesviridae (Choi, Lee, Lee, & Lee, 2021). CMV infection is usually acquired in early age and in people living in crowded places. Its seroprevalence increases with rise in age; and it ranges between 30-90.0% in developing countries. Incubation period of virus is 3-6 weeks, and after primary infection, the virus can be found in saliva, blood, and other body fluids in immunocompetent people. Now has become an extremely important pathogen as it is capable of producing congenital as well as acquired infections. WBC's and CD13-positive cells are the main reservoirs for harboring CMV(Pocock, 2018).

CMV is activated and transmitted through close contact with an infected person who is excreting a virus, through saliva, breastfeeding, pregnancy, blood transfusions, sexual contact, organ or stem cell transplant, immunosuppression, and disseminated malignancies. It infects T helper cells, changing their behavior. Human CMV is a widespread virus that can develop latency after primary infection and can be reactivated, especially during immunosuppressive periods. Activation of CMV might be associated with cellular response in the presence of foreign antigens (Forte, Zhang, Thorp, & Hummel, 2020). CMV infection may involve cells; endothelial, fibroblasts, smooth muscle and epithelial. Pathological hallmark is enlarged cell with inclusions bodies. CMV infection and complications are rarely seen among healthy individuals. The interaction of infectious particles with cellular receptors allows them to enter the cell (Anderson-Smits, Baker, & Hirji, 2020).

Diagnosed using a variety of approaches i.e. culturing, cytology and serological procedures to detect the presence of CMV antigens or nucleic acids in infected tissues. Due to late virus excretion, there is a chance that the primary infection may occur again in immunocompromised patient. PCR is considered as highly sensitive method as it detects small quantities of nucleic acid in various clinical samples, used for qualitative detection of CMV-DNA. The biology of CMV infection is quite complex but, in such cases, acquired immunity do not help to preventing from reinfection. Antibodies are essential to prevent from acquisition and spread of CMV with the help of seronegatives. In 2000, CMV was placed in the high priority list for the development of vaccine by the Institute of Medicine, US (Plotkin et al., 2020). There is currently no licensed HCMV vaccine, however recent clinical trials have made progress toward this aim.

Cytomegalic endothelial cells are responsible for causing infections in patients who receive blood transfusions such as patients of HD. Haemodialysis (HD) involves removal of excessive waste, the small particles (urea, creatinine etc.) and fluid outside a human body, used to treat and prevent kidney damage (Saravi & Mousavi, 2022). Blood is taken from the patient's body and pumped out by a

machine called a dialyzer during this procedure. Elevation of specific antibodies against the CMV in HD patients are related to ailments. In chronic and uremic patients on HD, infection caused by the CMV is the main reason for morbidity and mortality due to weakened immune response. There is a high burden of CKD in Pakistan according to the data revealed by a community-based study (Imtiaz & Alam, 2023). Systemic CMV infection occurs at a significant rate and the virus reactivates during severe deregulations of immune system (Khan, Hamid, & Lal, 2022).

Almost, 15-20% of persons with ≥ 40 years of age have a reduced GFR, which results in high prevalence of diabetes and hypertension, and this is one of the major reasons of end stage renal disease (Porrini et al., 2019). As a developing country, Pakistan nephrology units are not well developed but there is a noticeable awareness observed regarding these renal diseases and its co-morbidities such as CMV infection in HD patients since last decade (Naseem, Ayub, Shah, Ali, & Abidi, 2022). In chronic patients, the major cause of ailment and deaths is the infection caused by CMV. However, the investigations involved in normal encounter of viral infection among patients have been very limited. Therefore, the present study was aimed to determine the prevalence of CMV infection by sensitive techniques including; ELISA and quantitative PCR, in patients undergoing HD.

2. Methodology

2.1. Ethical Approval & Consent

The study received ethical approval from the Virtual University of Pakistan's Ethics Review Committee, Lahore.

2.2. Materials

For ELISA; A micro titration plate reader that can detect absorbance at 450 nm, Distilled water, 10 µl, 100 µl and 1 ml of pipettes, a semi-automatic pipette capable of delivering 100 µl, Automatic micro titration plate washer, Absorbent material designed for blotting of strips, incubator, HRP-conjugate and a TMB Reagent solution was used. For qPCR; we used TAN Bead® Nucleic Acid Extraction Kit, Auto Plates, Proteinase K, Elution Buffer and Spin tips. A micropipette No. 2, was procured from Aly. 12, Ln. 81, Longshou St., Taoyuan Dist., Taoyuan City 330, Taiwan Advanced Nanotech Inc. (R.O.C.) 2018-10-18 (revised) Version 2.1. Whole blood and serum specimens transported according to specific pathogen transit laws. During shipping, whole blood sample was kept at 2-25°C and we separated the serum within 6 hours. Serum samples transported at temperatures ranging from 2 to 8 degrees Celsius or frozen.

An interviewer administered close-ended proforma was used to collect data from the participants. The proforma included socio-demographic characteristics; family history of disease; co-illnesses; history off blood transfusion; anthropometric measurements; biochemical assays etc.

2.3. Method

2.3.1 Sampling and Selection Criteria

A Comparative cross-sectional study was carried out during the years of 2019-2020 and non-probability convenience sampling technique was used. In this study, a total of 150 blood samples were taken from patients undergoing HD.

Inclusion criteria includes; patients undergoing haemodialysis, with age ranges from 14 to 90 years, both male and female and belonging to any income class, caste or province of Pakistan. Exclusion criteria include; patients undergoing peritoneal dialysis with known cases of CMV. The data was collected from the dialysis unit. All patients undergoing HD were selected in a non-random way. The blood sample from patient was drawn in an EDTA vial and gel clotted vial after taking their consent and by properly guiding them about this action. Collected blood sample was stored at -20°C freezer for subsequent

Table 1 Reagent Components

Auto Plate	6	96 well plate with reagent buffers Elution Buffer 1.5ml
		Nuclease-Free Water
Proteinase K	1 ml	20 mg/ml Proteinase K, store at 4 ºC
Spin tips	96	Spin tip
Protocol	01	Instruction guide for user

analysis. Questionnaire was designed to record the clinical findings and the laboratory results of the patients.

2.3.2. CMV detection by ELISA

CMV IgM and IgG antibodies were detected by ELISA method. Principle of ELISA includes; Purified CMV antigen is coated on the surface of labelled microwells. When dilute serum is added to the wells, the CMV IgM specific antibody, if present, binds to the antigen. Everything that isn't bound is washed away with the water. The antibody-antigen combination is delivered to the HRP-conjugate, which binds to it. After rinsing away the excess HRP-conjugate, a TMB Reagent solution is added. The enzyme conjugate's catalytic action is halted at a predefined period. The intensity of the color produced is determined on the amount of IgM specific-antibody in the sample. The results were compared to the calibrator and controls in a parallel manner using a microwells reader. Procedure briefly includes following steps.

For Sample collection and handling some precautions were taken. Serum sample was utilized, and the typical venipuncture precautions was followed. Specimens were kept for two days at 2-8°C. Store at –20°C for extended periods. Specimens that had been hemolyzed or lipaemic were not used. Samples were not frozen and thawed repeatedly. All specimens and reagents-maintained at room temperature (~25°C) before use. We labelled the microwells for test. Diluted the serum samples with 1:101 dilution by adding 10 ul sample into 1 ml of Diluent buffer. Dispensed 100 ul diluted sample into each well. At 37°C, incubated for 45 minutes, then Washed the all well for 3 times through automated washer instrument. We filled each well with 100 µl of CMV-HRP Conjugate. Incubated for 45 minutes at 37°C, then Washed all the wells for 3 times through automated washer instrument. Then we added 100 µl of TMB Chromogenic Solution to each well using a dispenser. Incubated at room temperature for 15 minutes. Direct sunlight should be avoided. Using a dispenser, we poured 100 µl of Stopping Solution into each well. Lastly, we measured the absorbance of the solution in the wells with a microplate reader set to 450 nm within 30 minutes. Wavelength correction was possible, thus we set the instrument to dual wavelength measurement at 450 nm with background wavelength correction at 600 or 620 nm. For each control and unknown, we calculated the mean absorbance. For qualitative results; If the sample's absorbance is greater than the Cut-Off, it indicates positive for the presence of a

Table 2 Auto plate Component

Column	Buffer Solution	Volume
1/7	Lysis Buffer	600 µl
2/8	Washing Buffer 1	800 µl
3/9	Washing Buffer 2	800 ul
4/10	Washing Buffer 2	800 ul
5/11	Magnetic Beads	800 µl
6/12	Elution Buffer	80 l

specific IgM. Then we calculated the ratio between the sample's average OD value and the Cut-Off value. The following is a breakdown of the sample: If the ratio is more than 1.1, it is considered positive. If +/- 10% of the Cut-Off, it's unlikely. If the ratio is 0.9, it is negative. If we were not sure about the result, we repeated the test.

Limitations of the procedure may include; when only IgG antibodies are present in a serum sample taken during the late stages of illness, this approach may be negative and the relevance of serological data from immunocompromised individuals and newborn children is limited.

2.3.3. DNA extraction protocol and CMV detection by quantitative PCR

qPCR is suitable for in vitro diagnostic purposes. The Nucleic Acid Extraction Kit of high sensitivity used for clinical diagnostics and research purposes, was used to extract nucleic acids from a CMV. Samples treated with proteinase K prior to automated/semi-automated nucleic acid extraction process by Maelstrom 8.

We followed a protocol for DNA extraction as per instruction guide given with the kit. We, carefully removed the aluminium foil on the Auto Plate. In Auto Plate column #1/ #7, add 300 l serum or PBS solution and 10 l Proteinase K was used. The volume ratio of the mixture and the lysis buffer is approximately 300 l : 600 l. If altered, then may have an impact on performance. We mounted spin tips on Maelstrom 8 then placed Auto Plate to the plate holder of Auto stage. The missing corner of base faces toward the lower left. We selected a program “665-1/7” and parameters were set. When the program ends, we take out the Auto Plate carefully. Transferred the purified nucleic acid from column #6/#12 to a clean tube using a micropipette. No. 2. Then the used Auto Plate and spin tips were placed in the garbage can.

Figure 1 Cobas X 480 for quantitative detection of CMV virus

Nucleic acids extracted analyzed in downstream application, such as real-time PCR. The silicon dioxide coating on the magnetic beads can adsorb negative charged molecules to purify nucleic acid from samples. 300 l serum or PBS suspension as a sample was used. Some precautionary measures were taken. Components stored at room temperature (15-35 °C) until the expiration date on the package. At room temperature, proteinase K was transported. Proteinase K stored at 4°C once received. Vigorous shaking, plate exposure to environment and bleach-based detergent was avoided to maintain reagents stability and shelf life, as these can affect the extraction efficiency. We checked the integrity of the reagent plate before use, and mounted the spin tips in the proper position. Sterile consumables were used. Quantitative analysis was done lastly. Nucleic acid product purified by TAN Bead® nucleic acid extraction kit can perform quantitative analysis of specific genes by Q-PCR or qRT-PCR. It can also be used for detecting viral load other molecular detection analysis. All extracted sample results read by auto analyzer Cobas TaqMan.

The procedure of PCR is only suitable for in vitro diagnostic purposes. Precautionary measures may include. Placed the reagent plate in an oven (preheated 42–60ºC) for 5–10 minutes when the temperature is below 20. Avoid vigorous shaking, in order to avoid excessive formation of foam. Do not expose the reagent or plate to air after it has been opened. The evaporation would

Table 3 Age & prevalence of CMV infection by PCR

			QUANTITATIVE PCR VIRAL LOAD			
			Detected	**Not detected**	**Total**	**p-value**
Age (years)	**≤45**	**Count**	74	17	91	0.748
		Percent	81.3%	18.7%	100.0%	
	>45	**Count**	50	09	59	
		Percent	84.7%	15.3%	100.0%	
Total		**Count**	124	26	150	
		Percent	82.7%	17.3%	100.0%	

change the pH or affect the extraction efficiency. All of the reagents are clear and colorless. The presence of colored

reagent suggests contamination; we replaced the plate with a new one before continuing. We checked the integrity of the reagent plate before use, and mounted the spin tips in the proper position. We carefully removed aluminum foil to minimize splashing. To avoid nuclease contamination, we used sterile consumables. Because the reagent solution contains guanidine salt, do not use bleach-based detergent. Keep reagents away from our eyes, skin, and clothing and wear gloves and masks before handling.

For Specimen transportation, whole blood and serum specimens transported according to specific pathogen transit laws. During shipping, the whole blood sample maintained at 2-25°C and we separated the serum within 6 hours. Serum samples transported at temperatures ranging from 2 to 8 degrees Celsius or frozen.

For Quantitative analysis, Nucleic acid product purified by TAN Bead® nucleic acid extraction kit which can perform quantitative analysis of specific genes by Q-PCR or qRT-PCR. It can also be used for detecting viral load other molecular detection analysis. All extracted sample results read by auto analyzer Cobas TaqMan.

2.4. Statistical Analysis

The close-ended questionnaire included all possible responses with codes was utilized. Using these pre-assigned codes, data entry and analysis was done by using SPSS software version 26. Normality test was performed to assess the distribution of data. Data cleaning/ outliers/ bias removal was done as per protocol. Data was categorized on the CMV infection present and absent. The mean and standard deviation were used to explain numerical variables. Frequency was used to characterize categorical variables (percentage). P-value ≤0.05 was considered as significant.

3. RESULTS

3.1 Age

Total 150 patients undergoing HD were included in the study. Mean age of study population (n=150) was 42.28±13.63 years ranged from 14 to 84 years. Patients were categorized into two age groups i.e. ≤45 years and >45 years. Overall frequency of patients of age ≤45 years was 60.7% and patients of age >45 years 39.3%. Prevalence of CMV IgM positive cases was higher in age group ≤45 years (45.1%) than in >45 years (40.7%). Prevalence of CMV IgG positive cases was also higher in age group ≤45 years (74.7%) than in >45 years (67.8%), as shown in Fig. 2A. Total 82.7% patients were detected as positive for CMV infection. Prevalence of CMV infection was slightly lower in age groups ≤45 years (81.3%) than in >45 years (84.7%). However, the difference between groups was not significant (P-value 0.748), as shown in Table 3.

Table 4 Gender & prevalence of CMV infection by PCR

			QUANTITATIVE PCR VIRAL LOAD			
			Detected	Not detected	Total	p-value
Gender	**Male**	**Count**	81	19	100	0.593
		Percent	81.0%	19.0%	100.0%	
	Female	**Count**	43	07	50	
		Percent	86.0%	14.0%	100.0%	
Total		**Count**	124	26	150	
		Percent	82.7%	17.3%	100.0%	

Table 5a Haemoglobin & prevalence of CMV infection by PCR in Males

			QUANTITATIVE PCR VIRAL LOAD			
			Detected	Not detected	Total	p-value
Hb (g/dL)	**<12.5**	**Count**	71	16	87	0.708
		Percent	81.6%	18.4%	100.0%	
	≥12.5	**Count**	10	03	13	
		Percent	76.9%	23.1%	100.0%	
Total		**Count**	81	19	100	
		Percent	81.0%	19.0%	100.0%	

3.2 Gender

Overall frequency of male patients was 66.7% and female patients 33.3%. Patients were categorized in two groups based on gender i.e. male and female. Prevalence of CMV IgM positive cases was higher in males (45.0%) than in females (40.0%). While, the prevalence of CMV IgG positive as well as negative was equal for both genders as shown in Fig. 2B. Total 82.7% patients were detected as positive for CMV infection. Prevalence of CMV infection was slightly lower in male patients (81.0%) as compared to females (86.0%). However, the difference between groups was not significant (P-value 0.593), as shown in Table 4.

3.3 Haemoglobin Level

Mean of Hb level was 9.51±2.25 g/dL ranged from 4.9 to 14.6 g/dL. Overall frequency of male patients with anemia was 87.0% and 82.0% for females. Male patients were categorized into two groups i.e. Hb <12.5g/dL and Hb ≥ 12.5g/dL. Prevalence of CMV IgM positive cases was higher in group <12.5 (46.0%) than in ≥ 12.5 (38.5%). Prevalence of CMV IgG positive cases was also higher in <12.5 (73.6%) than in ≥ 12.5 (61.5%), as shown in Fig. 2C. Total 81.0% male patients were detected as positive for CMV infection. Prevalence of CMV infection was lower in ≥12.5 (76.9%) group as compared to <12.5 (81.6%) group. However, the difference between groups was not significant (P-value 0.708), as shown in Table 5a.

Female patients were categorized into two groups i.e. Hb <11.5g/dL and Hb ≥11.5g/dL. Prevalence of CMV IgM positive cases was higher in group Hb ≥11.5 (44.4%) than in Hb <11.5 (39.0%). Prevalence of CMV IgG positive cases was higher in <11.5 (73.2%) than in ≥11.5 (66.7%), as shown in Fig. 2D. Total 86.0% female patients were detected as positive for CMV infection. Prevalence of CMV infection was lower in <11.5 (82.9%) as compared to ≥11.5 (100%) group. However, the difference

Table 5b Haemoglobin& prevalence of CMV infection by PCR in Females

			QUANTITATIVE PCR VIRAL LOAD			
			Detected	**Not detected**	**Total**	**p-value**
Hb (g/dL)	**<11.5**	**Count**	34	07	41	0.325
		Percent	82.9%	17.1%	100.0%	
	≥11.5	**Count**	09	0	09	
		Percent	100.0%	0.0%	100.0%	
Total		**Count**	43	07	50	
		Percent	86.0%	14.0%	100.0%	

Table 6 TLC & prevalence of CMV infection by PCR.

			QUANTITATIVE PCR VIRAL LOAD			
			Detected	**Not detected**	**Total**	**p-value**
TLC (cmm)	**<4000**	**Count**	04	0	04	0.019
		Percent	100.0%	0.0%	100.0%	
	4000-10000	**Count**	78	23	101	
		Percent	77.2%	22.8%	100.0%	
	>10000	**Count**	42	03	45	
		Percent	93.3%	6.7%	100.0%	
Total		**Count**	124	26	150	
		Percent	82.7%	17.3%	100.0%	

between groups was not significant (P-value 0.325), as shown in Table 5b.

3.4 Total Leukocyte Count

Mean of TLC was 9318±6953 /cmm ranged from 3200 to 85000 /cmm. Frequency of patients with leucopenia was 2.7% and of leukocytosis 30.0%. Patients were categorized in three groups i.e. TLC >1000/cmm (leukocytosis), TLC 4000-1000/cmm (normal range) and TLC <4000/cmm (leucopenia). Prevalence of CMV IgM positive cases were higher in >1000 (55.6%) than in <4000 (50.0%) while low prevalence of CMV IgM positive cases (37.6%) was observed in 4000-1000. Prevalence of CMV IgG positive cases were higher in >1000 (80.0%) than in <4000 (75.0%) while prevalence of CMV IgM positive cases was (68.3%) in 4000-1000. Details are shown in Fig. 2E**.** Total 82.7% patients were detected as positive for CMV infection against TLC. Prevalence of CMV infection was higher in group <4000 (100.0%) as compared to >1000 (93.3%) and 4000-1000 (77.2%). Although, the difference between the groups was significant (P-value 0.019), as shown in Table 6.

3.5 Platelet Count

Mean of PLT count was 250233±112719 /cmm ranged from 21000 to 720000/cmm. Frequency of patients with thrombocytopenia was 16.7% and of thrombocytosis 7.3%. Patients were categorized in three groups i.e. PLT <150000/cmm (thrombocytopenia), PLT 150000-425000/cmm (normal range) and PLT >425000/cmm (thrombocytosis). Prevalence of CMV IgM positive cases were higher in 150000-425000 (48.2%) than in <150000 (32.0%) while low prevalence of CMV IgM positive cases (18.2%) was observed in >425000. Prevalence of CMV IgG positive cases were also higher in 150000-425000 (78.1%) than in <150000 (60.0%) while

Table 7 PLT count & prevalence of CMV infection by PCR

			QUANTITATIVE PCR VIRAL LOAD			
			Detected	Not detected	Total	p-value
PLT (cmm)	<150000	Count	20	05	25	0.923
		Percent	80.0%	20.0%	100.0%	
	150000-425000	Count	95	19	114	
		Percent	83.3%	16.7%	100.0%	
	>425000	Count	09	02	11	
		Percent	81.8%	18.2%	100.0%	
Total		Count	124	26	150	
		Percent	82.7%	17.3%	100.0%	

Table 8 Bilirubin total & prevalence of CMV infection by PCR

			QUANTITATIVE PCR VIRAL LOAD			
			Detected	Not detected	Total	p-value
Bilirubin total (mg/dL)	≤1.0	Count	83	16	99	0.764
		Percent	83.8%	16.2%	100.0%	
	>1.0	Count	41	10	51	
		Percent	80.4%	19.6%	100.0%	
Total		Count	124	26	150	
		Percent	82.7%	17.3%	100.0%	

low prevalence of CMV IgM positive cases (36.4%) was observed in >425000, as shown in Fig. 2F. Total 82.7% patients were detected as positive for CMV infection against PLT. Prevalence of CMV infection was higher in group

150000-425000 (83.3%) as compared to >425000 (81.8%) and <150000 (80.0%). Although, the difference between the groups was not significant (P-value 0.923), as shown in Table 7.

3.6 Bilirubin Total

Mean of bilirubin total was 1.10±1.07 mg/dL ranged from 0.2 to 8.0 mg/dL. Frequency of patients with hyperbilirubinemia was 34.0%. Patients were categorized in two groups i.e. ≤1.0 mg/dL and >1.0 mg/dL. As shown in Fig. 2G, the prevalence of CMV IgM positive cases was slightly higher in ≤1.0 (44.4%) than in >1.0 (41.2%). Prevalence for CMV IgG positive cases was also higher in ≤1.0 (75.8%) than in >1.0 (64.7%). Total 82.7% patients were detected as positive for CMV infection against Bilirubin total. Prevalence of CMV infection was higher in ≤1.0 (83.80%) as compared to >1.0 (80.4%). However, the difference between groups was not significant (P-value 0.764), as shown in Table 8.

3.7 Aspartate Transferase

Mean of aspartate transferase (AST) level was 53±69 IU/L ranged from 16 to 789 IU/L. Frequency of patients with raised AST level was 46.7%. Patients were categorized in two groups i.e. AST ≥40 IU/L and AST <40 IU/L. In Fig. 2H, it can be seen that prevalence of CMV IgM positive cases was higher in <40 (47.5%) than in ≥40 (38.6%). Prevalence of CMV IgG positive cases was also higher in <40 (76.3%) than in ≥40 (67.1%). In Table 9, Total 82.7% patients were detected as positive for CMV infection against AST. Prevalence of CMV infection was higher in <40 (86.3%) as compared to ≥40 (78.6%). However, the difference

Table 9 AST level & prevalence of CMV infection by PCR

			QUANTITATIVE PCR VIRAL LOAD			
			Detected	Not detected	Total	p-value
AST (IU/L)	**<40**	**Count**	69	11	80	0.306
		Percent	86.3%	13.8%	100.0%	
	≥40	**Count**	55	15	70	
		Percent	78.6%	21.4%	100.0%	
Total		**Count**	124	26	150	
		Percent	82.7%	17.3%	100.0%	

Table 10 ALT level & prevalence of CMV infection by PCR

			QUANTITATIVE PCR VIRAL LOAD			
			Detected	Not detected	Total	p-value
ALT (IU/L)	**<40**	**Count**	67	12	79	0.606
		Percent	84.8%	15.2%	100.0%	
	≥40	**Count**	57	14	71	
		Percent	80.3%	19.7%	100.0%	
Total		**Count**	124	26	150	
		Percent	82.7%	17.3%	100.0%	

Table 11 ALP level & prevalence of CMV infection by PCR

			QUANTITATIVE PCR VIRAL LOAD			
			Detected	Not detected	Total	p-value
ALP (IU/L)	**<140**	**Count**	14	03	17	1.000
		Percent	82.4%	17.6%	100.0%	
	≥140	**Count**	110	23	133	
		Percent	82.7%	17.3%	100.0%	
Total		**Count**	124	26	150	
		Percent	82.7%	17.3%	100.0%	

between groups was significant (P-value 0.306).

3.8 Alanine Transferase

Mean of ALT level was 58±94 IU/L ranged from 14 to 1092 IU/L. Frequency of patients with raised ALT level was 47.3% and 52.7% for normal range. Patients were categorized in two groups ALT <40 IU/L and ALT ≥40 IU/L. In Fig. 2I, Prevalence of CMV IgM positive cases was higher in <40 (48.1%) than in ≥40 (38.0%). Prevalence of CMV IgG positive cases was also relatively higher in <40 (78.5%) than in ≥40 (64.8%). In Table 10, total 82.7% patients were detected as positive for CMV infection against ALT. Prevalence of CMV infection was higher in <40 (84.8%) as compared to ≥40 (80.3%). However, the difference between groups was not significant (P-value 0.606).

3.9 Alkaline Phosphatase

Mean of alkaline phosphatase (ALP) level was 407±371 IU/L ranged from 69 to 3476 IU/L. Frequency of patients with raised ALP level was 88.7% and 11.3% for normal range. Patients were categorized in two groups i.e. ALP <140 IU/L and ALP ≥140 IU/L. Prevalence of CMV IgM positive cases was higher in

Table 12a Uric acid level & prevalence of CMV infection by PCR in Males.

			QUANTITATIVE PCR VIRAL LOAD			
			Detected	Not detected	Total	p-value
UA (mg/dL)	≤7.0	Count	51	10	61	0.569
		Percent	83.6%	16.4%	100.0%	
	>7.0	Count	30	09	39	
		Percent	76.9%	23.1%	100.0%	
Total		Count	81	19	100	
		Percent	81.0%	19.0%	100.0%	

Table 12b Uric acid level & prevalence of CMV infection by PCR in Females

			QUANTITATIVE PCR VIRAL LOAD			
			Detected	Not detected	Total	p-value
UA (mg/dL)	≤6.0	Count	18	05	23	0.225
		Percent	78.3%	21.7%	100.0%	
	>6.0	Count	25	02	27	
		Percent	92.6%	7.4%	100.0%	
Total		Count	43	07	50	
		Percent	86.0%	14.0%	100.0%	

Table 13 BSR & prevalence of CMV infection by PCR

			QUANTITATIVE PCR VIRAL LOAD			
			Detected	Not detected	Total	p-value
BSR (mg/dL)	≤150	Count	70	19	89	0.177
		Percent	78.7%	21.3%	100.0%	
	>150	Count	54	07	61	
		Percent	88.5%	11.5%	100.0%	
Total		Count	124	26	150	
		Percent	82.7%	17.3%	100.0%	

≥140 (72.2%) than in <140 (70.6%). Whereas, prevalence of CMV IgG positive cases was also relatively higher in ≥140 (43.6%) than in <140 (41.2%), as shown in Fig. 2J. In Table 11, Total 82.7% patients were detected as positive for CMV infection against ALP. There is only a slight difference of 0.3 among the prevalence of both groups. Prevalence of ALP <140 is (82.4%) while the prevalence of ALP ≥140 (82.7%). However, the difference between groups was not significant (P-value 1.000).

3.10 Uric Acid

Mean of uric acid level was 6.5±1.8g/dL ranged from 2.8 to 14.6g/dL. Frequency of male patients with hyperuricemia was 39.0% and of female patients with hyperuricemia was 54.0%. Male patients were categorized in two groups i.e. ≤7.0 mg/dL and >7.0 mg/dL. Prevalence of CMV IgM positive cases was slightly higher in ≤7.0 (45.9%) than in >7.0 (43.6%). Prevalence of CMV IgG positive cases was also higher in ≤7.0 (73.8%) than in >7.0 (69.2%). Overall prevalence against both type of antibodies is comparatively low in male patients with hyperuricemia as compared to the normal range, as shown in Fig. 2K. In Table 12a, total 81.0% male patients were detected as positive for CMV infection. Prevalence of CMV infection was higher in ≤7.0 (83.6%) as compared to >7.0 (76.9%). However,

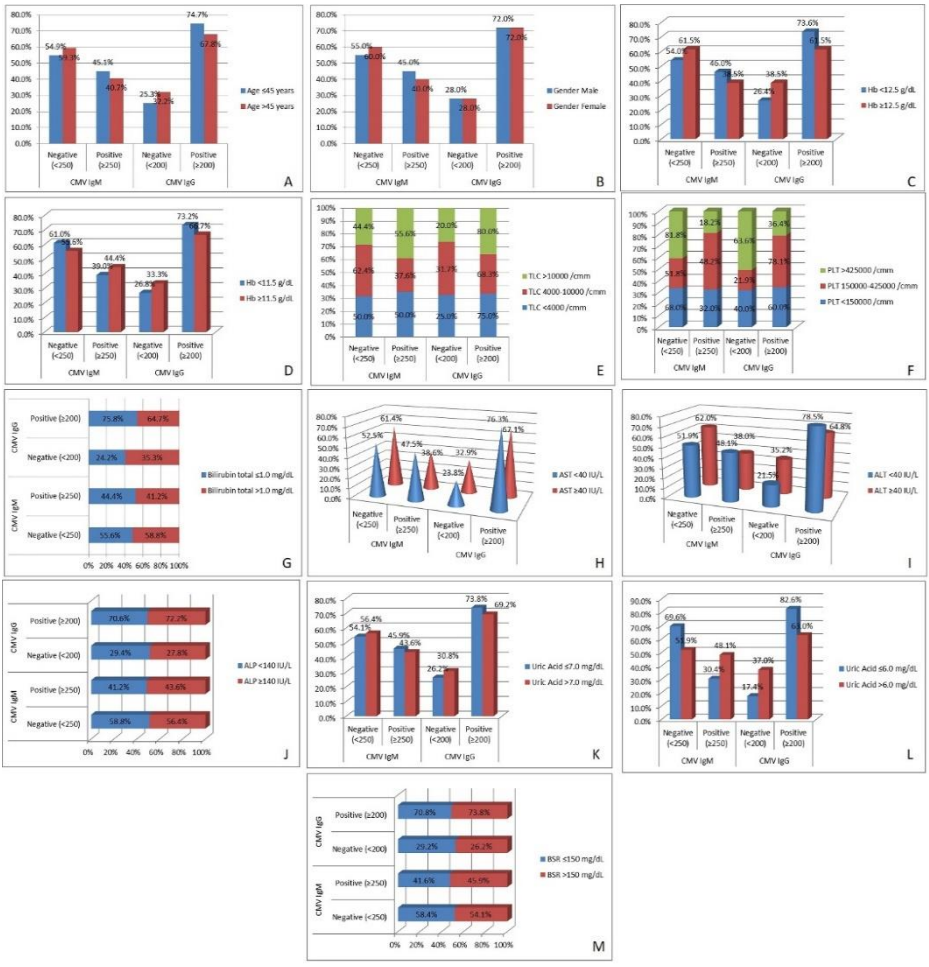

Figure 2 Prevalence of CMV infection by ELISA in relation to multiple parameters: **A.** Age & prevalence of CMV infection by ELISA; **B**. Gender & prevalence of CMV infection by ELISA; **C**. Haemoglobin & prevalence of CMV infection by ELISA in males; **D**. Haemoglobin & prevalence of CMV infection by ELISA in females; **E**. TLC & prevalence of CMV infection by ELISA; **F**. PLT count & prevalence of CMV infection by ELISA; **G.** Bilirubin total & prevalence of CMV infection by ELISA; **H.** AST level & prevalence of CMV infection by ELISA; **I**. ALT level & prevalence of CMV infection by ELISA; **J**. ALP level& prevalence of CMV infection by ELISA; **K**. Uric acid level & prevalence of CMV infection by ELISA in males; **L.** Uric acid level & prevalence of CMV infection by ELISA in females; **M.** BSR & prevalence of CMV infection by ELISA.

the difference between groups was not significant (P-value 0.569).

In Fig. 2L, female patients were categorized in two groups i.e. ≤6.0 mg/dL and >6.0 mg/dL. Prevalence of CMV IgM positive cases was higher in >6.0 (48.1%) than in ≤6.0 (30.4%). Whereas, the prevalence of CMV IgG positive cases was higher in ≤6.0 (82.6%) than in >6.0 (63.0%). In Table 12b, total 86.0% female patients were detected as positive for CMV infection. Prevalence of CMV infection was relatively higher in >6.0 (92.6%) as compared to ≤6.0 (78.3%).

However, the difference between groups was not significant (P-value 0.225).

Table 15 Prevalence of CMV infection by CMV IgG and Quantitative PCR

			QUANTITATIVE PCR VIRAL LOAD			
			Detected	Not detected	Total	p-value
CMV IgG (AU/mL)	Negative (<200)	Count	31	11	42	0.122
		Percent	73.8%	26.2%	100.0%	
	Positive (≥200)	Count	93	15	108	
		Percent	86.1%	13.9%	100.0%	
Total		Count	124	26	150	
		Percent	82.7%	17.3%	100.0%	

Table 14 Prevalence of CMV infection by CMV IgM and Quantitative PCR.

			QUANTITATIVE PCR VIRAL LOAD			
			Detected	Not detected	Total	p-value
CMV IgM (AU/mL)	Negative (<250)	Count	67	18	85	0.228
		Percent	78.8%	21.2%	100.0%	
	Positive (≥250)	Count	57	08	65	
		Percent	87.7%	12.3%	100.0%	
Total		Count	124	26	150	
		Percent	82.7%	17.3%	100.0%	

3.11 Plasma Glucose

Mean of plasma glucose level was 158±76 IU/L ranged from 49 to 510 IU/L. Frequency of patients with raised glucose level was 40.7% and 59.3% for normal range. Patients were categorized in two groups i.e. BSR ≤150 mg/dL and BSR >150 mg/dL. Prevalence of CMV IgM positive cases was higher in >150 (73.8%) than in ≤150 (70.8%). Prevalence of CMV IgG positive cases was also higher in >150 (45.9%) than in ≤150 (41.6%), as shown in Fig. 2M. In Table 13, total 82.7% patients were detected as positive for CMV infection against BSR. Prevalence of CMV infection was relatively higher in >150 (88.5%) as compared to ≤150 (78.7%). However, the difference between groups was not significant (P-value 0.177).

3.12 Cytomegalovirus Antibodies IgM

Mean of CMV IgM antibodies level was 323±210 AU/mL ranged from 109 to 987 AU/mL. Frequency of patients with positive CMV IgM level was 72.0%. Patients were categorized in two groups i.e. CMV IgM negative <250 and CMV IgM positive ≥250. Prevalence of CMV IgM positive cases ≥250 (43.3%) was lower than IgM negative <250 (56.7%). Details are shown in Figure 3A. Total 82.7% patients were detected as positive for CMV infection. Prevalence of CMV infection was relatively higher in CMV IgM Positive ≥250 (87.7%) as compared to Negative <250 (78.8%). The difference between groups was not significant (P-value 0.228).

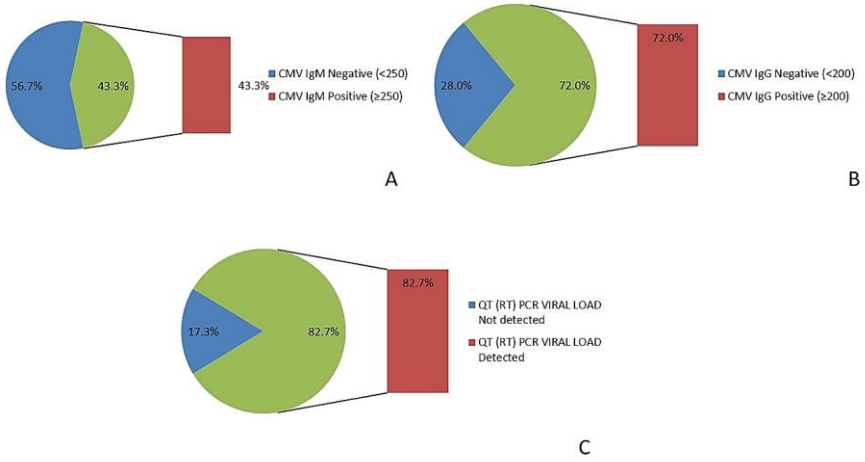

Figure 3 Prevalence of CMV. **A.** Prevalence of CMV IgG antibodies by ELISA. **B.** Prevalence of CMV IgM antibodies by ELISA. **C.** Prevalence of CMV infection by PCR.

3.13 Cytomegalovirus Antibodies IgG

Mean of CMV IgG antibodies level was 311±182 AU/mL ranged from 1.4 to 988.0 AU/mL. Frequency of patients with positive CMV IgG level was 43.3%. Patients were categorized in two groups i.e. CMV IgG negative <200 and CMV IgG positive ≥200. Prevalence of CMV IgG positive cases was higher in ≥200 (72.0%) than in IgG negative <200 (43.3%). Details are shown in Figure 3B. Total 82.7% patients were detected as

Table 16 Comparison of means between QUANTITATIVE PCR positive and negative cases

	QUANTITATIVE PCR VIRAL LOAD				
	Detected		**Not detected**		
	Mean	**Standard Deviation**	**Mean**	**Standard Deviation**	**p-value**
Age (years)	42.9	13.6	39.1	13.5	0.218
Haemoglobin (g/dL)	9.53	2.23	9.44	2.41	0.753
TLC (cmm)	9024.7	3257.8	10716.9	15279.2	0.134
PLT (cmm)	254258.1	108718.8	231038.5	130800.8	0.222
Bilirubin total (mg/dL)	1.1	1.0	1.2	1.5	0.879
AST (IU/L)	53.0	75.6	53.0	28.7	0.144
ALT (IU/L)	58.7	103.2	56.8	36.4	0.197
ALP (IU/L)	412.8	395.1	380.6	234.5	0.958
Urea (mg/dL)	177.1	68.2	170.7	64.8	0.548
Creatinine (mg/dL)	7.4	2.8	7.3	3.5	0.511
Uric acid (mg/dL)	6.6	1.9	6.4	1.8	0.559
BSR (mg/dL)	164.1	79.5	132.6	55.4	0.036
CMV IgG (AU/mL)	328.16	192.09	232.35	90.13	0.014
CMV IgM (AU/mL)	335.08	221.55	265.50	134.49	0.785

positive for CMV infection. Prevalence of CMV infection was relatively higher in CMV IgG Positive ≥200 (86.1%) as compared to Negative <200 (73.8%). Although, the difference between groups was not significant (P-value 0.122).

3.14 Quantitative PCR Viral Load

Frequency of patients with positive CMV viral load was 82.7%. Figure 4.14 shows the crux of all the results, which were obtained for CMV infections. 82.7% QT PCR viral load was detected while 17.3% remain undetected.

In Table 16, means of all variables are distributed along with their P-values. By considering the significant P-value 0.05, we can say that all the categories except BSR (mg/dL) and CMV IgG (AU/mL) were non-significant. Highest viral load was detected in PLT cmm (254258.1) while the lowest (1.1) was detected in Bilirubin total mg/dL. P-value ranges from 0.014 to 0.958. Details are shown in table 16.

4. Discussion

CMV is well-known for causing serious sickness in immunocompromised people; nonetheless, it is currently being recognized as a pathogen of emerging concern for patients with renal failure who are undergoing HD. The infections can strike at any time during a lifetime. CMV is a Herpes DNA virus with two strands, made up of an inner core, a capsid, and an envelope (Nahar, Hokama, & Fujita, 2019). Rise in specific antibodies against the CMV in HD patients are related to diseases like hepatitis, retinitis and pneumonitis. 60-90% of HD patients are seropositive, and this is mainly depending upon the age and socio-economic circumstances (Vilibić-Čavlek, Kolarić, Bogdanić, Tabain, & Beader, 2017). Among methods for CMV diagnosis for CMV antigens or antibodies and viral nucleic acid, PCR and ELISA are considered highly sensitive. The choice for diagnosing IgM and IgG tests for CMV infection depends primarily upon the duration of HD that determined the duration of infection. Noticeable awareness observed regarding the renal diseases and its co-morbidities in relation to CMV infection in HD patients since last decade in Pakistan especially among chronic patients. However, the researches based on normal encounter of viral infections in HD patients have been quite limited, therefore, present study aimed to explore the CMV prevalence by ELISA and qPCR in HD patients.

This study was conducted to estimate the seroprevalence of CMV infections among the renal failure patients undergoing HD with the average dialysis treatment time in this study was approximately two years. In our study, prevalence positive CMV viral load (82.7%) was markedly higher than prevalence of CMV IgG positive cases (72.0%) and almost twice higher than CMV IgM positive cases (43.3%). Results were compared between CMV IgG and CMV IgM antibodies using the sensitivity and specificity measures. The study comprised of 150 individuals categorized into two groups of antibodies against CMV infections with age of study population ranged from 14 to 84 years. At first, prevalence of CMV infections was calculated based on gender and a total of 82.7% patients were detected as positive. Prevalence of CMV infection was slightly lower in age groups ≤45 years (81.3%) than in >45 years (84.7%) (Nikolich-Žugich et al., 2020). Significantly lower prevalence of CMV antibodies in males (81.0%) as compared to females (86.0%) was found. Patients samples were subjected to check the prevalence based on blood Haemoglobin concentration. Prevalence of CMV infection was lower in males ≥12.5 (76.9%) as compared to <12.5 (81.6%) (White et al., 2019). Whereas in females, prevalence of CMV IgM positive cases was higher in group Hb ≥ 11.5 (44.4%) and CMV IgG positive cases was higher in <11.5 (73.2%) (Saleh, Abd Al-Hussien, & Ighawish, 2018).

Leukocyte population reduced mostly during CMV disease and this reduction is independent of any immunosuppressive therapy. Leukopenia is considered as one of the clinical manifestations of CMV infections. The prevalence of CMV infection by ELISA based on TLC was also explored and were checked separately for each concentration as shown in figures. Total 82.7% patients were detected as positive for CMV infection against TLC. While prevalence of CMV infection was higher in group <4000 (100.0%) as compared to >1000 (93.3%) and 4000-1000 (77.2%) (Liang, Famure, Li, & Kim, 2018). PLT and prevalence of CMV was calculated based on three concentrations. Prevalence of CMV IgM and IgG positive cases were higher in normal range 48.2% and 78.1% while thrombocytopenia has low prevalence of 18.2% and 36.45 for CMV IgG. Prevalence of CMV infection was also calculated by the concentration of bilirubin. Positivity of IgM (44.4%) and IgG (75.8%) cases were higher in ≤1.0 than in >1.0 mg/dL.

The frequency of patients with raised AST level was 46.7%. Prevalence of CMV infection was higher in <40 age group (86.3%) as compared to ≥40 (78.6%). Prevalence of CMV infection was higher in <40 (84.8%) as compared to ≥40 (80.3%) (Ye & Zhao, 2017). ALP levels were also calculated, 82.7% patients had raised ALP levels. Prevalence of CMV IgM and IgG positive cases was 72.2% and 43.6% in ≥140 IU/L that indicates that there was a high risk for these patients to get CMV as compared with the normal range. Patients with elevated levels of uric acid are more prone to CMV as well as many other disorders (Lopez Diaz & Macuyama Saavedra, 2022). Frequency of male patients with hyperuricemia was 39.0% and frequency of female patients with hyperuricemia was 54.0%, thus females are at higher risk. CMV seropositivity is somehow associated with many indicators of glucose regulation (Contreras et al., 2019). In the present study, frequency of patients with raised glucose level was 40.7% and 59.3% for normal range and total 82.7% patients were detected as positive for CMV infection against BSR. Prevalence of CMV IgM positive cases was higher than in CMV IgG positive. Prevalence of CMV antibodies i.e. IgM and IgG were checked separately. Prevalence rates of CMV IgM positive and IgG positive were relatively higher as compared to the negative ones. Therefore, the overall seroprevalence of CMV infection among these patients was 99% using CMV IgG and 100% using CMV IgM ELISA (Adane & Getawa, 2021). Figure 3 shows the crux of all the results, which were obtained for CMV infections. 82.7% viral load was detected by PCR (Arapović et al., 2020).

By considering the significant P-value 0.05, we can say that all the categories except BSR (mg/dL) and CMV IgG (AU/mL) were non-significant. Highest viral load was detected in PLT cmm (254258.1) while the lowest (1.1) was detected in Bilirubin total mg/dL. P-value ranges from 0.014 to 0.958. In the present study, CMV PCR positive test was detected in 124 patients while 26 patients were negative with CMV. In these patients, the base rate of CMV IgM seroprevalence was 99. CMV IgM levels have been observed to peak 1–3 months after primary infection and then drop to a low level for 18–39 weeks. The rise in IgM titer may occur before the rise in IgG titer after the initial commencement of illness.

Our findings were in agreement with the previous studies as there was a gradual increase in the seroprevalence of patients above 40 years, prevalence rate is higher in young females and the seroprevalence of CMV IgG and IgM detected was similar to the findings reported in previous studies. Although, many studies have focused on various aspects of the topic, but none of them deal with this particular research idea. No such study was found in literature in which prevalence of CMV is calculated based on Uric acid levels. Despite of the

shortage of data about the sensitivity and specificity of IgG and IgM antibodies, the study will contribute for further research in CMV diagnosis particularly for the patients with renal failure undergoing HD. In addition, the method by which the sample was collected helps to minimize the liability of selection procedures.

5. Conclusion and Research Contributions

CMV infection associated rising cases and mortalities demands some novel early sensitive diagnostics tools with better efficiency. HD linked immunocompromised patients are at higher risk of acquiring CMV infection and related mortality or morbidity in Pakistan. Current study was focused on CMV antigens or antibodies detection via qPCR and ELISA for molecular detection of viral nucleic acid. As a developing country, Pakistan nephrology units are not well developed and CMV infection in HD patients were common. Present study exploited the prevalence of CMV infection by ELISA and quantitative PCR, at department of Molecular Biology, Virtual University of Pakistan, Lahore during 2019-20. 150 patients were enrolled based or specific criteria via non-probability sampling technique and an interviewer administered close-ended proforma was used to collect data from the patients. Blood specimen was drawn and whole blood sample was used to estimate Hb, TLC and PLT count. Serum sample was used to estimate bilirubin total, AST, ALT, ALP, urea, creatinine, uric acid and glucose levels. CMV IgM and IgG antibodies were detected by ELISA method and CMV viral load by quantitative PCR. Data was analyzed by using SPSS v.26.

The study concluded that prevalence of positive CMV viral load (82.7%) was markedly higher than prevalence of CMV IgG positive cases (72.0%) and almost twice higher than CMV IgM positive cases (43.3%). Screening for CMV infection by commonly used ELISA method may give false negative results. Therefore, patients undergoing HD should be screened for the presence of CMV infection by quantitative PCR.

6. Limitations and Future Recommendations

As screening for CMV infection by ELISA method may give false negative results. Therefore, a well-designed cross-sectional validation study with large sample size measuring the diagnostic accuracy of quantitative PCR method versus ELISA method is recommended for future.

Furthermore, dialysis duration related risk, immunosuppressive therapies related outcome bias, and comorbidities are key cofounders, independently influence clinical outcomes but were not fully addressed in our analysis. Thus, future studies should be focused on more comprehensive data collection and statistical data adjustments in order to address these issues. Despite these constraints, the study provides important insights and we plan to address these address confounders in subsequent research by incorporating more detailed clinical data and performing multivariable analysis to better isolate the effects of primary variables of interest.

7. DECLARATION OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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A Unique and Green Method Designed for the Detection of Minute Quantities of Zinc in Real and Natural Specimens

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Abstract

In this study, we have developed an easy and fast spectrophotometric procedure to analyze zinc in trace amount using a reagent 1-(2-thiazolylazo)-2-naphthol (TAN) in surfactant cetyltrimethylammonium bromide (CTAB) solution. Zinc reacted with 1-(2-thiazolylazo)-2-naphthol to give bis[1-(2-thiazolylazo)-2-naphthol]zinc. Designed spectrophotometric method has been of great significance as using the micellar system instead of toxic, expensive and time taking extraction method. This method presented an improved detecting efficacy, sensitivity and coefficient of molar absorption. The Sandel's sensitivity and molar absorption coefficient were determined to be 4.5 ngcm^{-2} and ε 1.96×10^4 L mol^{-1}cm^{-1} at λ_{max} 581.4 nm, respectively. The 1:2 ratio was observed for Zn-[TAN]$_2$ development. Linear calibration curve was obtained within 0.12-4.0 µg mL^{-1}. At pH 6.5, complex formation occurred and remained stable for 24 hrs. Our recommended procedure was applied successfully for the investigation of Zinc from various alloy, ecological, pharmaceutical and biological specimens.

Keywords: Zinc; Cetyltrimethylammonium Bromide; 1-(2-Thiazolylazo)-2-naphthol; Complexation

1. Introduction

The detection of trace metal ions is of paramount importance across diverse domains, including biology, environmental science, and industrial applications. Metallic ions such as Cu (II), Zn (II), Fe (II), Co (II), and Mn (II) are not only integral to various biochemical processes but are also essential for the physiological functioning of living organisms (May, Linder, & Williams, 1977), whereas metal ions, such as arsenic (As), cadmium (Cd), lead (Pb), and Mercury (Hg), are poisonous to living systems at certain concentrations (Stankovic, Kalaba, & Stankovic, 2014), while valuable metals are hazardous at much greater concentrations. Zinc (Zn) ion is vital for entire systems of animals, plants and humans, participates in numerous living roles (Natasha et al., 2022), its chief function is the formation of numerous enzymes and co-enzymes. It behaves as both metalloenzyme and enzymes activator. It is involved in the production of deoxyribonucleic acid (DNA) and ribosomal ribonucleic acid (RRNA) (Shankar & Prasad, 1998). They are essential for the nerve systems and DNA synthesis (Gower-Winter & Levenson, 2012). Zinc imparts core role in person's blood scattered 3%, 75% - 85% and 12% - 22% in leukocytes, erythrocytes and plasma respectively

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(Reddy & Reddy, 2011). Zn is commercially available and is utilized in the pharmaceutical industry as vitamins, balms, eye drops and lotions (Mohiuddin, 2019). Zinc (II) ions contain vitamins utilized in nutrients as a supplement for vitamins, which is essential to improve tissue formation, heal wounds, traumatic injury, sex and hair growth (Grada & Phillips, 2022). Zn (II) ion deficiency indicates forfeiture of sexual hormones and hair growth (Wong, Thomas, Merkus, Zielhuis, & Steegers-Theunissen, 2000), diminution in collagen production, procrastinating soothing of injuries, and detrimental DNA creation (Hara et al., 2017). Its deficiency also causes growing obstruction, lesser food proficiency, ulcers, and skin scaling (Prasad, 1995). Though, high consumption of zinc is unsafe but its moderate consumption is vivacious to healthy life. Zinc sulphate capsule (220 mg) causes nausea and vomiting (Reddy & Reddy, 2011). It is utilized in dry batteries, photoengraving, protection against corrosion and lithography (Prkić, Giljanović, Petričević, Brkljača, & Bralić, 2013).

During the spectrophotometric study of metallic ions, the reaction of metals with a chelating agent and their dissolution in a surfactant system offers an efficient and rapid approach for metal ion analysis (Soomro & Shar, 2014).

A range of spectrophotometric methods have been introduced as alternatives to the conventional solvent extraction technique, incorporating the use of surfactants (Yun & Choi, 2000). The micellar systems because of solubility of different metallic complexes show enhancement in the analysis of metal ion techniques (Olkowska, Polkowska, & Namieśnik, 2012). The micellar system is acquaint with improve molar absorption and sensitivity and substitute old extraction solvent method. Numerous spectrophotometric approaches are available for zinc estimation with different chelating agents. Some most recent established spectrophotometric approaches of analysis have least sensitivity and selectivity (Allan, 1961; Ghaedi et al., 2009; Rao, Balaji, Rao, Babu, & Naidu, 2002; Sammut et al., 2008). We have developed a novel, robust, efficient, and effective method for detecting zinc (II) in various real and natural samples using the 1-(2-thiazolylazo)-2-naphthol (TAN) chelating agent in cetyltrimethylammonium bromide (CTAB) aqueous micellar solutions. Previous research has not investigated zinc (II) ions using TAN in CTAB aqueous micellar systems. This newly developed method demonstrates improvements in several analytical characteristics, including the limit of detection, molar absorptivity coefficient, Beer's law range, and Sandell's sensitivity when applied to CTAB surfactant solutions. The proposed procedure has significant applications in natural, environmental, industrial, and medicinal studies and is widely recognized worldwide for its accessibility, simplicity, and eco-friendliness.

2. Material and Methods

Ultra-violate visible spectrophotometer, FT-IR spectrophotometer, pH/conductivity meter and Atomic absorption spectrophotometer were used.

2.1. Reagent Preparation

The CTAB solution of 0.02 M was made by using 7.28 g of CTAB in measuring flask of 1000 ml and deionized water was added until the final volume reached the mark (Korai et al., 2022). Zn (II) 1000 μgL^{-1} solution was made from its salt zinc nitrate $Zn(NO_3)_2$ (Merck Darmstadt, Germany) in graduated flask (Abdolmohammad-Zadeh & Sadeghi, 2009). The 4×10^{-3} M solution of reagent TAN was made up with addition of 0.50 g of TAN containing 25 ml of methanol into volumetric flask of 500 mL and CTAB 0.02 M was added to make up the

volume (Korai et al., 2022). The solutions of buffers from pH 1 to 10 were made as per the procedures by adding suitable quantities of both HCl-KCl equimolar 0.2 M for 1-4 pHs, CH_3COOH-CH_3COONa equimolar 0.2 M for 5-6 pHs, KH_2PO_4-NaOH equimolar 0.1 M for 6.5-8 pHs and 0.025 M sodium borate - 0.1 M HCl for 9-10 pHs solutions (Perrin, 2012).

2.2. Zinc (II) metal ion detection by general procedure

Zinc ion concentrations ranging 0.06-10 μgmL^{-1}, 2 mL (5×10^{-4} M) solution of TAN, 2 mL of different pHs buffer solutions and 1-2 mL (0.02 M) solution of CTAB were allowed to mix in a 10 mL volumetric flask and distilled water was added to make the final volume. Zinc metal ion absorbance at optimal settings for the formation of metal complex was detected at specific λ_{max} using a UV-vis spectrophotometer.

2.3. Detection of Zn (II) from alloy specimens

The 0.1g of each alloys specimens were mixed 50-60 mL HCl 6.0 M and 30% H_2O_2 3-5 mL volume and heated and added distilled water to get the 1 L diluted solution. 10 mL volume of each solution specimens was placed in graduated flagon of 250 mL volume separately and added the distilled water to the mark. The specimens were reacted with 5×10^{-4} M TAN at 6.5 pH in 0.02 M CTAB, then zinc-complexes absorbances were recorded. Results are presented in Table 3 & 6.

2.4. Zn (II) analysis from a tap water sampling

Tap water specimen was collected from the Sukkur area. The sample was then subjected to filtering with filter paper of 0.45 µm and 1mL of concentrated HNO_3 was added to acidify the solution to prevent precipitations. Metal of zinc (II) was spiked into the specimen, 2 mL of 5×10^{-4} M TAN, 2 mL of buffer of 6.5 pH and 2 mL of 0.02 M CTAB were mixed in, after that, the Zn-$[TAN]_2$ complex absorption was recorded, results are presented in Table 4.

2.5. Estimation of Zn (II) from pharmaceutical specimen

A powdered tablet was digested by adding the 10 mL volume of 70% conc. perchloric acid and heated to dryness. The residues were dissolved with the mixing of 5 mL volume of 0.1 M HCl, solution was filtered and placed in calibrated flask of 1000 mL volume and added distilled water up to mark. The specimen was reacted with 5×10^{-4} M TAN at 6.5 pH in 0.02 M CTAB, then zinc-complex absorbance was recorded. Results are presented in Table 5.

2.6. Investigation of zinc (II) from vegetable specimen

The specimen of vegetable *caymopsis psoralides* locally known as Guwar was gathered from the vegetable market in Pano Akil, Pakistan, washed and oven dried at 110 ^{0}C. The specimen was powdered using mortar and pestle. 5 g of powdered specimen was taken and digested by mixing 2 mL of H_2O_2 30% and 10 mL of HNO_3 conc. on hot plate heating until decreased to 2-3 mL volume then filtered it and added distilled water up to 25 mL volume and adjusted the required pH. Then, the 5 mL of specimen solutions were transferred to a graduated beaker and 2 mL of 5×10^{-4} M TAN, 2 mL of buffer of 6.5 pH and 2 mL of 0.02 M CTAB were mixed and absorbance was recorded. Results are presented in Table 5.

2.7. Analyzing Zn (II) content from environmental water sampling

Samples containing 1 liter of wastewater from different locations in Pano Akil, Sukkur district, and Mirpur Mathelo industrial area, Ghotki district, Pakistan, were collected. Specimens were subjected to filtering and acidification with addition of H_2O_2 (30% concentrated) 2 mL and HNO_3 (conc.) 4 mL. Then the resulting solutions were pre-concentrated by heating in an oven at 110 °C to obtain 25 mL of solutions finally. Then, the

specimen solutions were transferred to a graduated beaker and 2 mL of 5×10^{-4} M TAN, 2 mL of buffer of 6.5 pH and 2 mL of 0.02 M CTAB were mixed in, after that, the Zn-$[TAN]_2$ complex absorption was measured. Results are presented in Table 5.

2.8. Detection of zinc (II) from environmental specimens

The 1-5 g of each oven dried ecological solid specimens were mixed 50-60 mL HCl 6.0 M and 30% H_2O_2 3-5 mL volume and heated to dryness. The residues were dissolved with the mixing of 10 mL volume of 1 M HCl, solutions were filtered and placed in calibrated flask of 1000 mL volume and added distilled water up to mark. The specimens were reacted with 5×10^{-4} M TAN at 6.5 pH in 0.02 M CTAB, then zinc-complexes absorbances were recorded. Results are presented in Table 6.

3. Results and Discussion

3.1. Spectrophotometric investigation of Zinc using TAN

Zinc produced coloured metal chelate when it was reacted with reagent 1-(2-thiazoylazo)-2-naphthol (TAN) in the presence of surfactant CTAB. The derivatizing agent TAN is tridentate having three lone pair of electron donating sites as given in Figure 1. The surfactant solution was employed for metal chelate solublization as to estimate the zinc metal ions in minute quantities.

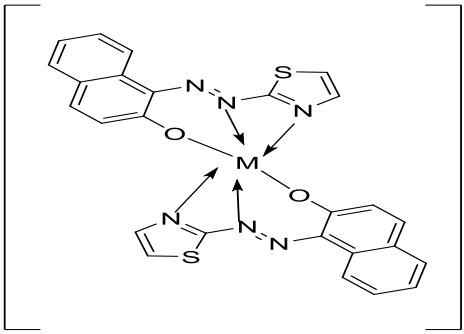

Figure 1 Structure proposed for metal (II)-$[TAN]_2$ chelate

3.2. UV-Vis spectra

Complexing reagent 1-(2-thiazoylazo)-2-naphthol solution displayed orange-red color and exhibited absorption sharp peak at λ_{max}488.5 nm in region of UV-Vis spectrum due to electronic transition $\pi\rightarrow\pi^*$, in fact, the charge transfer took place from ligand to ligand L→LCT. The complexing reagent (TAN) UV/Vis spectrum is given in Figure 2(a).

The chelate Zn (II)-$[TAN]_2$ spectrum in UV-vis region represented enhanced longer absorbance bands for N=N and N=C bathochromic sharp band shifted to 92.9 nm from $\pi\rightarrow\pi^*$. L→MCT took place to unoccupied zinc (II) dπ orbital and occupied chelate pπ orbital at λ_{max}581.4 nm. It was perceived that the oxygen of O-H and nitrogen of N=C groups on de-protonation contributed in developing bonds for the creation of Zn (II)-$[TAN]_2$ chelate as presented in Figure 2(b).

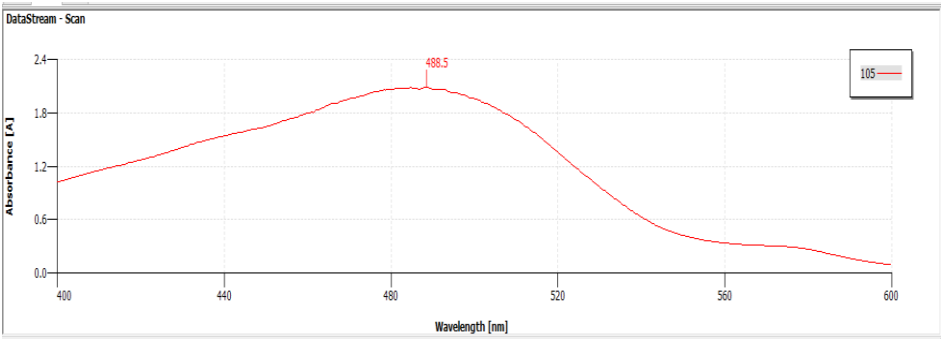

Figure 2(a) UV-Vis spectrum of complexing reagent TAN at λ_{max}488.5 nm

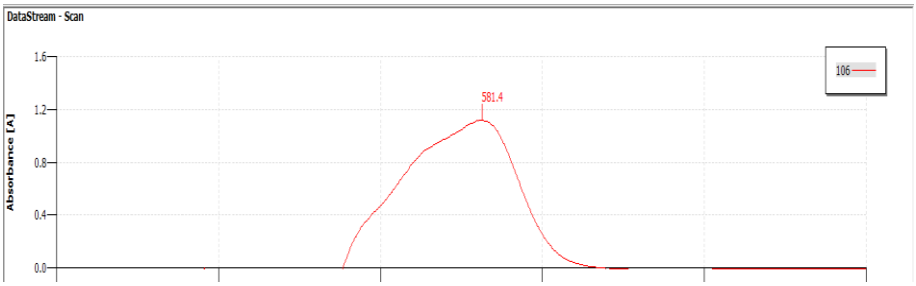

Figure 2(b) UV-vis spectrum of Zn (II)-$[TAN]_2$ complex at λ_{max}581.4 nm

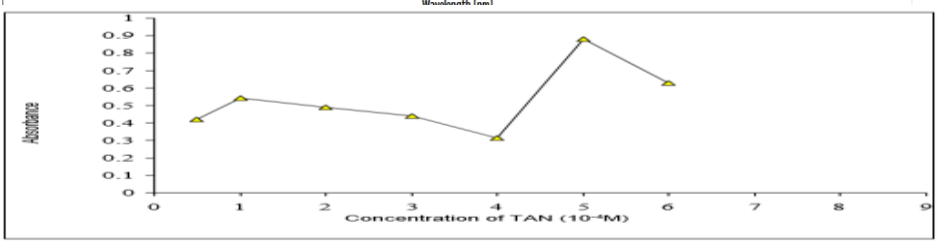

Figure 3. Influence of comlexing agent TAN concentration

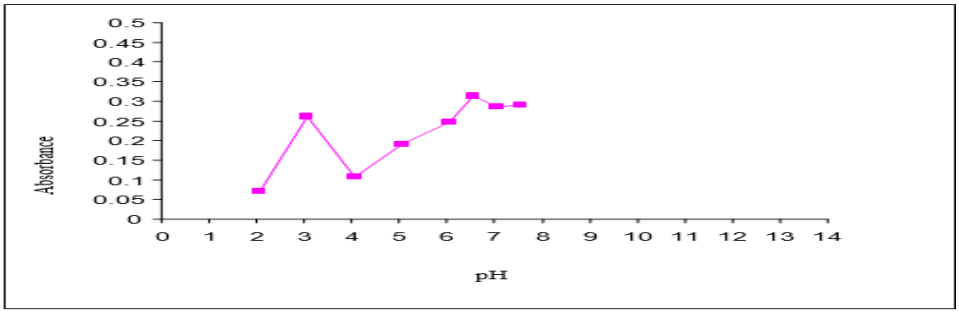

Figure 4. pH graph for the Zn-(TAN) complex in CTAB

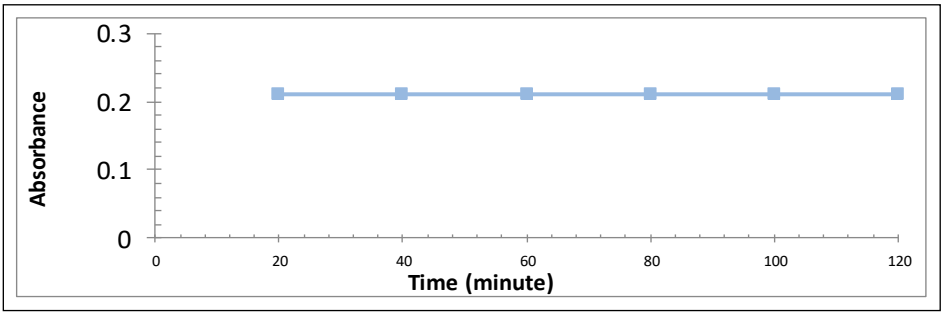

Figure 5. Metal -TAN complex stability

3.3. Ratio of Metal to complexing reagent

The Molar ratio method was employed for the investigation of composition of metal chelate (Malik & Rao, 2000). Metal zinc to reagent TAN ratio was obtained as 1:2 for the development of Zn (II)-$[TAN]_2$ complex (Table 1).

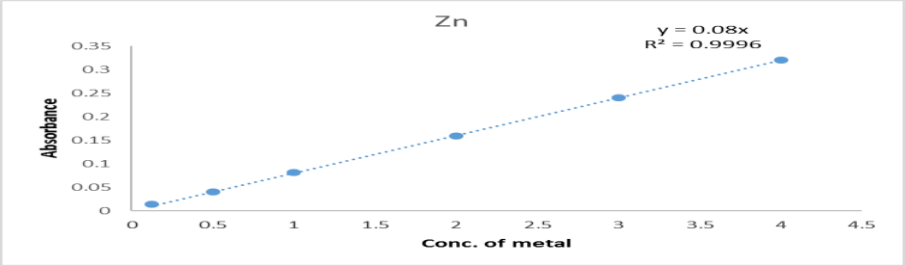

Figure 6 Calibration graph of Zn (II)-$[TAN]_2$

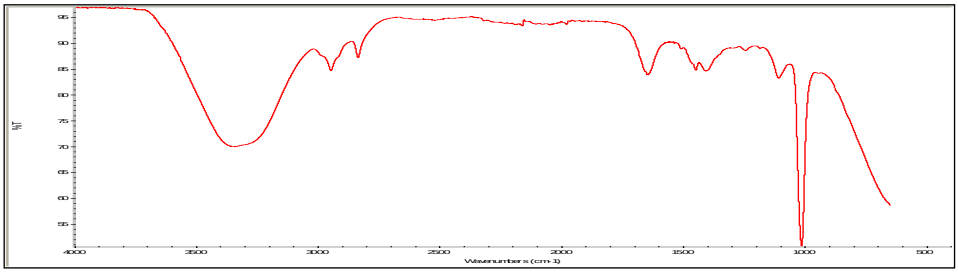

Figure 7(a) FT-IR spectrum of complexing reagent TAN

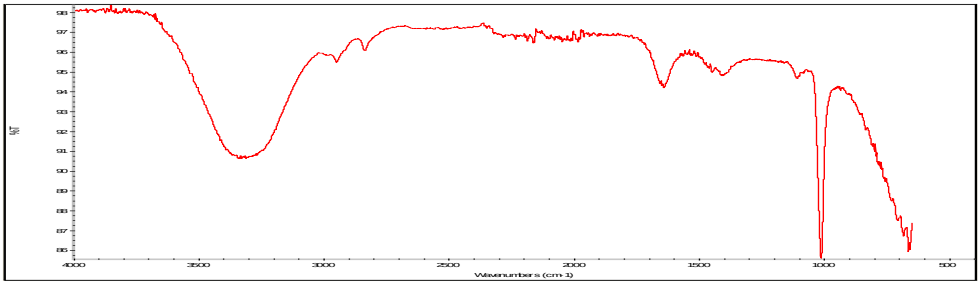

Figure 7(b) FT-IR spectrum of Zn (II)-$[TAN)_2$ compound

3.4. Effect of concentrations of surfactant cetyltrimethylammonium bromide (CTAB) and complexing reagent TAN

The surfactant CTAB 0.02 M solutions of different quantities were investigated for complexation and absorbance maxima was observed when CTAB 0.02 M 2 mL volume was employed with fixed quantity of 2 mg/L of metallic ion. The TAN complexing reagent concentrations from 0.5 to 8×10^{-4} M influenced on complexation of metal with chelating agent, absorbances were noted using different concentrations and absorbance maxima for metal chelate was observed at 5×10^{-4} M concentration that was taken as optimum condition and was utilized throughout the research as given in Figure 3 and Table 1.

3.5. pH and time influence

pH influence on retrieval extraction in general investigation was carried with fixed values of other parameters. Optimal pH for zinc was detected 6.5 and it was selected for more research as given in Figure 4 and Table 1.

Metal chelate formation was examined; the complexation was quick and offered fixed maximum absorbance at room temperature and remained unchanged until 2hrs as displayed in Figure 5.

Table 1 Characteristic parameters for Zn-TAN complex

Parameters	Zinc
Molar absorptivity	1.96×10^4 $Lmol^{-1}cm^{-1}$
Limit of detection	5.1 $ngmL^{-1}$
Beer's law range	0.12-4.0 μgmL^{-1}
Concentration of TAN	5.0×10^{-4} M
Surfactant CTAB	2.0 mL
pH	6.5
L:M	2:1
Sandell's sensitivity	4.5 $ngcm^{-2}$
Wavelength (λ_{max})	581.4 nm
R^2	0.9996

3.6. Zn (II)-$[TAN]_2$ Calibration

The graph of calibration for Zn (II) at λ_{max}581.4 nm offered linear concentration ranges 0.12-4.0 mg/mL with R^2 0.9996 intercepting through zero as displayed in Figure 6.

3.7. Sandell's sensitivity, limit of detection and coefficient of molar absorptivity

Linear calibration curve revealed the mean co-efficient of molar absorptivity for zinc (II) at λ_{max} 581.4 nm that was measured as 1.96×10^4 $Lmol^{-1}cm^{-1}$. Limit of detection was noted as 5.1 $ngcm^{-2}$. Sandell's sensitivity was observed to be 4.5 $ngcm^{-2}$ (Table 1). Results obtained were analogous to the results indicated in literature reported.

3.8. Influence of different ions in Zinc analysis

The solutions with zinc (II) of 10 µg and varied contents of several ions (cations & anions) were prepared and charted process. The limit of interference of ion was set as the ratio in which ± 2% deviation in absorbance was calculated. Bi (III), Al (III), Mn (II), Ba (II), Mg (II), Ca (II), nitrate, carbonate, phosphate and chloride ions were with no any interfering effect as a lowest 100:1 mass ratio (Reddy, Reddy, & Reddy, 2011). More influences were noted as in Table 2.

Table 2 Foreign ions interference

Added ions	Tolerance Range (µg)
Mo (VI)	50
Bi (III)	60
As (III)	65
Pd (II); Sn (IV); Fe (III)	80
Co (II); Ni (II); Cu (II)	100
Al (III)	150
Pt (IV); Au (III)	200
Mg (II)	300
Cr (VI); Ba (II)	400
Sodium sulphate	500
Ascorbic acid; Sodium potassium tartate	1000
Ammonium phosphate; Sodium thiosulphate	2000
NaF	2100
$NaNO_3$	3100
Sodium citrate; Dimethylglyoxime	5000
KCl	8100
Thiourea; Potassium thiocyanate	10000
KI	11000

3.9. FT-IR spectra

The reagent TAN FT-IR spectrum offered bands of absorption for *v* (O-H), *v* (C-H)(C-N) groups and aromaticity at 3342.70, 2948.39 and 1651.39 cm^{-1} respectively as presented in Figure 7(a). Bands ranges 1500 cm^{-1} to 4000 cm^{-1} indicated the stretching whereas 550 cm^{-1} to 1500 cm^{-1} indicated finger print area. Complex Zn-$[TAN]_2$ exhibited bands of absorption for *v* (O-H), *v* (C-H)(C-N) groups and aromaticity at 3332.92, 2942.38 and 1655.13 cm^{-1}. These different bands proposed M-O-H and M-

Table 3 Analysis of Zn (II) from alloys specimens

(%) Certified Alloys	Metallic ions	Metal existent	Metal obtained	% RSD	% Relative errors	% Recoveries
BCR-191	Zinc (II)	1.5 µg	1.38 µg	0. 79	0.13	92.00
Bronze PUC-2	Zinc (II)	4.55%	4.31	0.53	0.24	94.73

Table 4 % recovery of Zn (II) in tap H_2O

Metals	Mixed (µg/mL)	obtained (µg/mL)	% Recovery
Zn (II)	1.75	1.69	96.57

N bonding in Zn-$[TAN]_2$ as displayed observed for metallic ion in H_2O, and % in Figure 7(b).

Table 5 Examination of Zn (II) from real and pharmaceutical specimens

Specimens	Analytes	Present method (μgmL^{-1})	% RSD	AAS procedure (μgmL^{-1})	% RSD	%Recoveries
Surbex Z tablet	Zn (II)	22.41	0.3	22.45	0.4	99.3
Vegetable sample (mg/kg) (field vetch) *caymopsis psoralides*	Zn (II)	15.37	0.51	15.42	0.46	98.3
Municipal water (mg/L)	Zn (II)	13.78	0.7	14.1	0.5	99.0
Industrial Wastewater (mg/L)	Zn (II)	27.5	0.2	28.0	0.4	97.5

Table 6 Study of Zn (II) content from certified material and environmental specimens

Certified Reference Material				
(%) Certified	**Metallic ions**	**Present (µg/g)**	**Obtained (µg/g)**	**%Recovery**
IRMM-3702	Zinc (II)	14	13.98	99.85

Environmental specimens		
Specimens	**Present method (mg/g) Zinc obtained**	**A.A.S method (mg/g) Zinc obtained**
Soil near Pano Akil	24.71 ± 0.4	24.80 ± 0.5
Fly ash near Mirpur Mathelo	126.2 ± 3.2	126.5 ± 3.25

3.10. Accuracy and precision

IRMM-3702 certified reference material and Bronze PUC-2 alloy were investigated to validate the methodology. In addition, to obtain the result reliability of the current procedure was ensured via percentage recovery process by usage of identified quantity of ion in specimens, recovery was 96.57% (Table 3-6).

3.11. Validation of method

This recommended procedure was applied for estimation of zinc (II) metal in natural, alloy, real, medicinal, environmental and biological specimens. Obtained results presented good agreements with the results of AAS as displayed in Table 5-6. This procedure

was compared with existing procedures. Developed suggested procedure has offered improvement in molar absorptivity, limit of detection, linear calibration range and Sandell's sensitivity than previous stated procedures (Table 7).

Table 7 Comparison of detection method for of zinc (II) with TAN

Metal	Complexing agents	Procedures/Remarks	References
Zinc (II)	PPT	ϵ 1.6×10^4 $Lmol^{-1}cm^{-1}$ at λ_{max}430 nm, Sandell's sensitivity $4.085\times10^{-3}\mu g/cm^{2,}$ Linear range up to 6.0 µg/mL.	(Sarma, Kumar, Reddy, Thriveni, & Reddy, 2006)
Zinc (II)	BPT	ϵ 1.8×10^4 L $mol^{-1}cm^{-1}$ at λ_{max}430 nm, limit of detection 0.064 μgmL^{-1}, Beer's law range 0.26 to 2.61 µg/mL.	(Reddy et al., 2011)
Zinc (II)	HPHOPD	ϵ 0.156×10^3 L $mol^{-1}cm^{-1}$ at λ_{max}415 nm, linear calibration range 1 to 20 ppm.	(Tekale, 2012)
Zinc (II)	DBHQ	ϵ 1.62 x 10^5 L mol^{-1} cm^{-1} at λ_{max}391nm, LOD 5.0 µg/L, Linear range 0.02-4.0 mg/L.	(Islam & Ahmed, 2013)
Zinc (II)	TAN	ϵ 1.96 x 10^4 L/mol.cm at λ_{max}581.4 nm, linear calibration range 0.12-4.0 µg/mL, Sandell's sensitivity 4.5 ng/cm^2.	Present method

4. Conclusion

The developed recommended procedure was employed to detect trace amounts of zinc (II) metal ions using the complexing reagent TAN in a CTAB surfactant, as opposed to the previous solvent extraction method. This new procedure is more efficient, quick, sensitive, secure, and environmentally pleasant for detecting zinc (II) ions in minute quantities. The proposed method demonstrates significant improvements in molar absorptivity, limit of detection, linear calibration range, and Sandell's sensitivity compared to the previously described techniques, as presented in Table 7. The obtained results were compared with those from certified reference materials, the AAS method, official techniques, and statistically validated at a 95% confidence level, demonstrating comparability. This recommended procedure has been successfully applied for the estimation of zinc (II) in a variety of samples, including natural, alloy, real, medicinal, environmental, and biological specimens, at trace levels.

5. Statements & Declarations

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5.2. Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

5.3. Availability of data and material

Corresponding authors will provide data on request.

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Characterization of Municipal Solid Waste Generating at UVAS Ravi Campus, and its Conversion into Compost Using *Lumbricus Terrestris*

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Abstract:

A predictable production of municipal solid waste ranges between 1.3 and 1.9 billion tons per year all over the world and has become an increasing problem day by day while being expected to increase almost 2.2 billion tons per year by 2025. Today established nations are facing a big problem in the management of municipal solid waste with the development of industries. 25 million tons of the solid waste is produced every year. Vermicomposting is a simple and cost-effective method to decompose several types of crop residues and agro industrial wastes. The aim of the present study was to evaluate the nutrient status of compost and vermicompost prepared by two earthworm species namely *Eisenia fetida* and *Lumbricus terrestris*. It has been estimated that one kg of the organic matter is consumed by earthworm in a day. Castings of the earthworms are rich in phosphorus, nitrogen, potassium, calcium and magnesium. Proximate analysis indicates that vermicompost contains 1-1.5 percent phosphorus, 1.5-2 percent potassium and 2.5-3 percent nitrogen. Depending upon the quality of the substrate vermicompost also contains hormones, vitamins and enzymes. These all characteristics are improved by increasing enzymatic and biological activities of earthworms. Vermicomposting is like receiving gold from garbage to improve richness of the soil. Ultimate product of the vermicomposting comprises more hormone like complexes, nutrients such as Mg, Ca, K, N, P, vitamins, enzymes and fewer heavy metals as compare to traditional composting.

Keywords: Traditional compost, Vermicompost, Characterization of MSW, Earthworms, Physiochemical parameters, Heavy metals

1. Introduction

Vermicomposting is an effective technique to recycle on-farm wastes into valued manure. Vermi-composting is one such organic technique that can be used for the conversion of the bakery industry slush into manure (Yadav et al., 2015). In vermicomposting earthworms are used for the production of vermicompost. Compost is a mixture of worm castings, organic material, humus, living earthworms and other micro-organisms. Conversion of massive organic wastes into low volume nutrient enriched end product is called composting (Yousefi et al., 2013). With-in the rapid developments of industries production of municipal solid waste (MSW) has been increased and its quantity has been increasing day by day. Structures and quantities of MSW differ from country to country as well as from region to region and district to district. Income level and socio-economic structures of people are the main reasons of these variations.

Moisture content and calorific value analyses are important parameters for waste characterization (Ozcan et al.,

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2016). Waste that is generated from industries, municipal activities, and human settlements is generally considered as municipal solid waste. Municipal solid waste also generates from public areas such as leftover from streets and gardens and leftover from domestic such as glass, newspaper and metals and leftover from waste water treatments such as manure and mud. Waste of paper industries and domestic wastes are also significant in terms of vermicomposting (Amouei et al., 2017). Vermicomposting technology has industrial as well as domestic value in numerous countries like USA, Italy, Japan and Canada. In 1970 Canada stared the technology of vermicomposting (Singh and Sinha 2022). 75 tons of the organic waste is being treated per week. Aoka Sangyo Co. Ltd is a well-organized company of japan which process 1000 tons of organic waste from food and pulp industries per month. Italy and Philippines also have well organized vermicomposting plants to process organic wastes (Nath and Singh 2016; Bhat et al., 2018). Castings of the earthworms are rich in phosphorus, nitrogen, potassium, calcium and magnesium. Proximate analysis indicates that vermicompost contains 1-1.5 percent phosphorus, 1.5-2 percent potassium and 2.5-3 percent nitrogen. Beneficial microbes such as bacteria, protozoa, fungi and actinomycetes are also present in vermicompost (Hoitink and Fahy 1986).

There are about 3000 species of earthworms all over the world. Species of the earthworms generally used for vermicomposting includes *Eisenia feotida, Lamito mauritti, Perionyx excavates, Eisenia andrie, Lumbricus terrestris, Lumbricus rubellus* and *Dravida willsi* (Arancon et al., 2004). Municipal solid waste needs ratio 1:1 of nitrogen and carbon for active composting (Follet et al., 1981). Ultimate product of the vermicomposting comprises more hormone like complexes, nutrients such as Mg, Ca, K, N, P, vitamins, enzymes and fewer heavy metals as compare to traditional composting (Grappelli et al., 1987). Vermicompost comprises of macronutrients as well as micronutrients (Pigatin et al., 2016). For effective vermicompost progression ideal temperature extended from 18-67°C, electrical conductivity oscillated from 0.70-80 μscm^{-1}, pH 5.9-8.3, moisture content 10.6-80% (Singh et al., 2013).

2. Materials and Methods

Study was conducted at Ravi Campus, University of Veterinary & Animal Sciences Pattoki C block. Municipal waste generated at C Block was collected and characterized. For the biotransformation of waste mixtures, local species of earthworms present in Pattoki was used due to its improved survival potential in municipal waste (Yadav and Garg 2009).

2.1. Collection and Segregation of Municipal Solid Waste

Municipal solid waste (MSW) was collected from C block on daily basis for a period of one month and manually segregated into different categories such as organic matter, recyclable material (paper and plastic) and disposable material. The collected waste was weighed on daily basis and the compostable material (organic portion) was transferred to compost bins for predecomposition of the waste.

2.2. Calorific Value and Moisture Content Analysis

Laboratory equipment, such as a bomb calorimeter was used for the measurement of calorific (heating) value of the collected waste (Ozcan et al., 2016). From the categorized wastes almost 5 kg waste sample was randomly nominated for calorific value analysis. In standardized samples moisture content (MC) of solid wastes was checked according to the TS10459 standard.

Table 1 Total amount of Municipal Solid Waste generated at C block.

Sr. No	Components	Weight (kg)
1	Amount of MSW per day	50 kg
2	Amount of MSW per week	350 kg
3	Amount of MSW per month	10500 kg
4	Proportion of organic waste in MSW per day	40 kg
5	Proportion of organic waste in MSW per week	280 kg
6	Proportion of organic waste in MSW per month	1200 kg
7	Proportion of recyclable waste per month	150 kg
8	Amount of waste in each bin	23 kg
9	Weight of empty bin	2 kg
10	Initial weight of each bin	25 kg
11	Final weight of each bin	11 kg
12	Average %age reduction of waste	60 %

2.3. Predecomposition and Introduction of Earthworms

Vermicomposting was performed in four plastic bins having at least a diameter of 12 inches and a height of 24 inches. Weight of the empty bin was 2 kg. For predecomposition of waste, five kg of the segregated organic waste was used in each bin and mixed with cow dung and poultry waste. Traditional compost (control) was prepared identically but without earthworms, with weekly turning for aeration. Both systems maintained similar moisture (60–70%) and initial C:N ratios (25–30) to ensure comparable baseline conditions. Two bins served as untreated controls, containing the same organic waste mixture (kitchen waste, cow dung, and brown waste) but no earthworms. These control bins were managed identically to experimental bins (same layering, moisture maintenance via water sprinkling, and biweekly turning) to isolate the effect of earthworms (*Lumbricus terrestris* and *Eisenia fetida*) on decomposition efficiency. Due to logistical constraints and alignment with prior vermicomposting studies (Yadav and Garg 2009), this preliminary investigation used two experimental and two control bins. Future studies should expand the sample size to enhance statistical robustness. First layer of soil (4 kg) and second layer of cow dung (4 kg) was added until bin is ½ full. Third layer was made of kitchen waste (8 kg) and forth layer of brown waste (4 kg). All the material was covered with soil. Total weight of the bin was 22 kg with 1:2:1 of brown waste, kitchen waste and cow dung. Water was sprinkled over the material. After allowing the waste to pre-decompose for twenty days to decrease C: N ratio and to release heat produced through early decomposetion in all bins, *Lumbricus terrestris* at the rate of 100 g per bins were introduced in the experimental bins. The waste material was regularly checked for temperature, pH, moisture content and Electrical conductivity (EC). The compost was turned after every fifteen days and water was sprinkled as per requirement (Singh et al., 2011). Compost samples were collected after every thirty days and analyzed for quality parameters until the compost is fully prepared. After 4 months samples of mature compost were harvested from bins to analyze physico-chemical parameters. Compost samples were thoroughly sieved through 0.2 mm sieve.

2.4. Physico-Chemical Analyses

Throughout the vermicomposting and composting progression temperature (°C),

Table 2 Physical parameters of Traditional compost (Tc) vermicompost prepared by *Lumbricus terrestris* (V1) and vermicompost prepared by *Eisenia fetida* (V2) observed after every seven days.

Date	**Temperature (◦C)**			**pH**			**Electrical conductivity** ($mScm^{-1}$)			**Moisture Level (%)**		
	Tc	**V 1**	**V 2**	**Tc**	**V1**	**V2**	**Tc**	**V1**	**V2**	**Tc**	**V 1**	**V2**
07-08-2019	32	31	30	7.0	7.0	7.0	3.64	3.64	3.64	Wet	Wet	Wet
14-08-2019	30	29	28	7.0	7.0	7.0	3.64	3.64	3.64	Wet	Wet	Wet
21-08-2019	31	30	29	7.0	7.0	7.0	3.65	3.64	3.64	Wet	Wet	Wet
28-08-2019	30	30	30	7.0	6.5	6.5	2.70	2.69	2.69	Wet	Wet	Wet
05-09-2019	29	28	29	6.5	6.5	6.5	3.35	3.34	3.34	Wet	Wet	Wet
12-09-2019	28	29	29	6.5	6.5	6.5	2.70	2.70	2.68	Wet	Wet	Wet
19-09-2019	29	30	30	6.5	6.5	6.5	2.90	2.80	2.75	Wet	Wet	Wet
26-09-2019	28	27	27	5.5	5,5	5,5	2.85	2.80	2.80	Wet	Wet	Wet
03-10-2019	27	26	26	6.5	6.0	6.0	3.20	3.34	3.34	Wet	Wet	Wet
10-10-2019	26	26	26	7.0	6.5	6.5	3.20	3.3	3.3	Wet	Wet	Wet
17-10-2019	25	24	24	6.5	6.0	6.0	3.20	3.42	3.48	Wet	Wet	Wet
24-10-2019	26	25	24	5.5	6.o	6.0	3.64	3.54	3.56	Wet	Wet	Wet
01-11-2019	25	24	24	5.5	6.5	6.5	3.64	3.60	3.60	Wet	Wet	Wet
08-11-2019	25	25	25	6.5	7.0	7.0	3.61	3.61	3.61	Wet	Wet	Wet
15-11-2019	24	24	23	7.0	7.0	7.0	3.66	3,65	3.65	Wet	Wet	Wet
22-11-2019	24	23	23	6.5	6.5	6.5	3.65	3.69	3.69	Dry	Dry	Dry
30-11-2019	23	22	21	6.5	6.5	6.5	3.70	3.70	3.70	Dry	Dry	Dry
08-12-2019	20	21	20	6.5	6.5	6.5	3.70	3.70	3.70	Dry	Dry	Dry
Sig. Level	0.643			0,987				0.985				

pH, and moisture content (%) were noted after 7 days with the help of digital meter. For effective vermicompost progression ideal temperature extended from 18-67°C, electrical conductivity oscillated from 0.70-80 μscm^{-1}, pH 5.9-8.3, moisture

Table 3 Physico-Chemical parameters of mature compost and vermicompost.

Parameters	**Compost**	**Vermicompost prepared by *Lumbricus terrestris* (V1)**	**Vermicompost prepared by *Eisenia feotida* (V2)**	**Significance Level**
Temperature (◦C)	22◦C	25◦C	27	0.00
Moisture level (%)	Dry	Dry	Dry	
pH	7.0	7.0	7.0	0.62
EC ($mScm^{-1}$)	3.7	3.5	0.11	0.00
Colour	Dark brown to black	Dark brown to black	Dark brown to black	
Odour	Absence of foul odour	Absence of foul odour	Absence of foul odour	
Particle Size	Minimum 90% material passed through 4.0 mm IS sieve	Minimum 90% material passed through 4.0 mm IS sieve	Minimum 90% material passed through 4.0 mm IS sieve	
Bulk density	< 1.0	0.8	0.9	0.57
Organic Carbon (%)	13	17	18	0.00
Nitrogen (%)	1.42	1.60	1.78	0.00
Phosphorus (%)	0.92	1.05	2.08	0.00
Potassium (%)	0.33	1.44	1.65	0.00
Carbon to nitrogen ratio (%)	11	18	20	0.00

content 10.6-80% (Singh et al., 2013). Likewise, EC was determined using Hanna's edge EC (HI2003). Chemical examination was done by means of Walkey-Black technique to quantify the organic carbon of the sample. Kjeldhal apparatus was used to determine the N content of the sample and K content was determined by flame photometric technique. P was determined by bomb calorimeter. Heavy metals such as Ni, Zn and Pb were estimated by using Atomic Absorption Spectrophotometer.

2.5. Statistical analysis:

The data was analyzed for any significant differences in three groups by using one way ANOVA with software Statistical Package for the Social Sciences (IBM SPSS) version 26.. ANOVA was computed to test the level of significance between three compost samples with respect to nutrient parameters.

3. Results and Discussion

Municipal solid waste (MSW) was collected from C block on daily basis for a

Table 4 Amount of Heavy Metals observed in Compost and Vermicompost.

Heavy Metals	**Compost**	**Vermicompost prepared by *Lumbricus terrestris* (V1)**	**Vermicompost prepared by *Eisenia fetida* (V2)**	**Significance Level**
Zinc (ppm)	412	405	398	0.19
Lead (ppm)	65	60	52	0.00
Nickle(ppm)	19	13	12	0.00

period of one month and manually segregated into different categories such as organic matter, recyclable material (paper and plastic) and disposable material. The collected waste was weighed on daily basis and the compostable material (organic portion) was transferred to compost bins for predecomposition of the waste. In this study preparation of compost and vermicompost was observed by using municipal solid waste generated at UVAS C block Pattoki. Kitchen waste, brown waste and green waste were used as a source of organic waste. (Singh et al., 2018) described that 9.1 million tons of the compost is prepared every year from the municipal waste. Highest rate of the weight mass was noticed in the form of organic waste which was 85 percent. Organic solid waste has 70 percent moisture content and calorific value 2417.4 kcal. These outcomes are according to the conclusions of (Ozcan et al., 2016). They find out moisture content as 71 percent and calorific value as 2518.5 kcal. The collected waste was weighed on daily basis and the compostable material (organic portion) was transferred to compost bins for predecomposition of the waste (Table 1).

Soil survey instruments were used to detect the physical parameters of the samples (Table 2). The pH of the compost and vermicompost was decreased from 7.0 to 6.5. Reduction in the pH makes nutrients readily available to the plants.

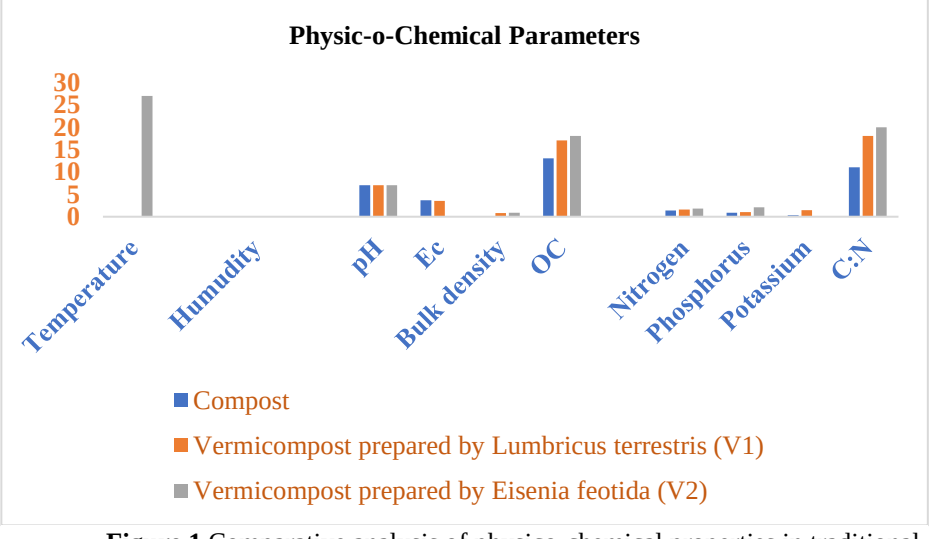

Figure 1 Comparative analysis of physico-chemical properties in traditional compost (Tc) and vermicomposts produced by Lumbricus terrestris (V1) and Eisenia fetida (V2).

This relates to the findings of (Sakthivel et al., 2017). Particle size of the matured compost is very important for nurseries as proper drainage is improved by particles > 13 mm. Electrical conductivity showed variation in the results of vermicompost prepared by *Eisenia fetida* and *Lumbricus terrestris*. In present study temperature of the final product was in the range of 22-27°C. Electrical conductivity of the final product was in the range of 0.11-3.7 µscm^{-1}. These findings were according to the conclusions of (Singh et al., 2013).

For effective vermicompost progression ideal temperature extended from 18-67°C, electrical conductivity oscillated from 0.70-80 µscm-1, pH 5.9-8.3, moisture content 10.6-80% (Singh et al., 2013) Level of significance was 0.00 for temperature and EC, 0.62 for pH & 0.57 for bulk density. Mean square was 0.33 for temperature, 0.13 for pH, 0.93 for EC & 0.03 for bulk density by applying One way ANOVA. By applying chemical parameters, amount of organic carbon was higher in vermicompost as compare to traditional compost. Table 3 and figure 1 indicates the physical & chemical parameters of the mature compost and vermicompost. In present study, carbon to nitrogen ratio of the compost was 11. Vermicompost prepared by *Lumbricus terrestris* has a carbon to nitrogen ratio such as 18 and vermicompost prepared by *Eisenia fetida* has a C:N ratio such as 20. Carbon to nitrogen ratio reduces with the passage of time. In present study these results were according to the findings of (Paul et al., 2019). Vermicomposting increases potassium, phosphorus and nitrogen and also reduces carbon to nitrogen ratio. When these results were compared with the vermicompost prepared by *Eisenia fetida*, more significant results were obtained. Level of significance for all chemical parameters was 0.00. Mean square was 0.33 for OC 0.00 for nitrogen 0.01 for potassium & 0.33 for C: N by applying One way ANOVA. Table 4 and figure 2 indicates the amount of heavy metals in compost and vermicompost. Concentration of heavy metals was reduced in vermicompost as compare to traditional compost. Concentration of heavy metals was low in vermicompost prepared by *E. feotida* as compare to vermicompost prepared by *L. terrestris*.

It was concluded that *E. feotida* has maximum resistance to heavy metals as compare to *L. terrestris*. This relates to the results of (Wang et al., 2017). Level of significance was 0.19 for zinc, 0.00 for lead and nickle. Mean square was 21 for zinc, 1.55 for lead and 0.66 for nickle by applying One way ANOVA. Highest ability to accumulate heavy metals was noticed in *Eisenia* species (Suleiman et al., 2017). Following order was noted for the accumulation of heavy metals Zn>Pb>Ni.

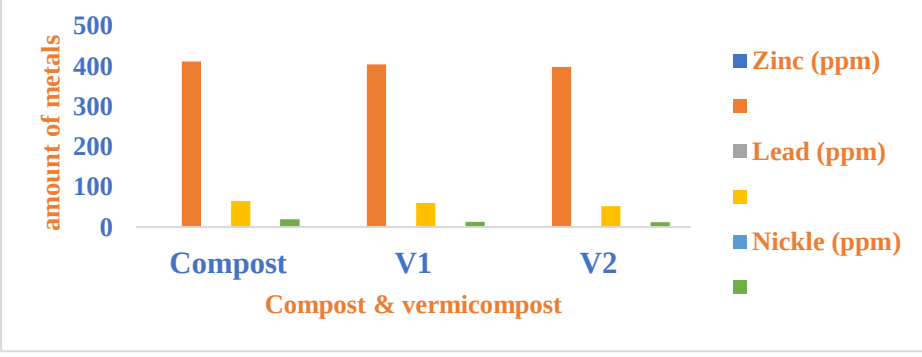

Figure 2 Concentration of Heavy Metals (Zinc, Lead & Nickle) observed in Compost and Vermicompost.

The findings of (Suleiman et al., 2017) were quite different from our results. They conclude that Zn>Ni>Pb. Toxicity and concentration of heavy metals was low in vermicompost (Wang et al., 2017). The significant reduction of heavy metals (Zn, Pb, Ni) in vermicompost, particularly with Eisenia fetida, results from earthworm-mediated bioaccumulation in tissues and metal complexation with organic matter in castings (Wang et al., 2017). E. fetida demonstrated superior metal resistance, accumulating up to 78% of Pb in chloragogenous tissues (Suleiman et al., 2017), while humic acid complexes in castings reduced metal bioavailability by 23-30%. However, metal concentrations in spent earthworms (Zn: 412 ppm) exceeded EPA safety thresholds, necessitating secure disposal methods such as anaerobic digestion or phytoremediation with *Brassica juncea* to prevent ecological risks (Bhat et al., 2018).

4. Conclusion

Vermicompost prepared by an earthworm species such as *Eisenia fetida* contains high level of phosphorus, potassium, organic carbon and nitrogen as compare to traditional compost and vermicompost prepared by an earthworm species such as *Lumbricus terrestris.* While this study focused on physico-chemical parameters, future work could assess microbial communities and enzymatic activity (e.g., urease, cellulase) to elucidate biological mechanisms driving nutrient transformations.

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6. Statements & Declaration

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6.2. Conflict of Interest

The authors have declared there is no conflict of interest.

6.3. Ethical Approval

Not applicable.

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