Nat. Volatiles & Essent. Oils, 2021; 8(6): 1630-1641

NVEO
Natural Volatiles &
Essential Oils

The Qualitative Evaluation Of Some Amino Acids In Urine Of Children With Nephrotic Syndrome

Haider Abed Zaid Jassim [1] , Thaer M. M. Al-Rammahi[*1] , Qahtan M. A. Al-Obaidy[2] , Riyadh Abd-Alrasool Hamid Henwa[3] and Haneen Haleem Shablawi[1]

[1]Department of Chemistry, College of Science, University of Kerbala, Karbala, Iraq

[2]Karbala Teaching Hospital for Children, Karbala, Iraq

[3]Head of Biochemistry and Hormone Laboratory, Al-Hussain (AS) Medical City, Karbala, Iraq

Abstract

Nephrotic syndrome is one of the common pathological cases in children in Karbala, Iraq. The disorder of amino acids metabolism can be considered as a major cause of the nephrotic syndrome, especially in children, and disorders of protein and amino acids metabolism can include conditions that occur when the body is unable to break down certain amino acids or their production, which leads to the accumulation of toxic substances for some substances or a deficiency in the production of beneficial compounds. This work was included children with nephrotic syndrome (n=50) and healthy children (n=50) within the age group (2-10 years). Samples (urine and blood) were collected for the participants of the study (patients and healthy volunteers).Thin-layer chromatography (TLC) technique was used to identify the amino acids in urine samples, which were conducted in the study (50 healthy, 50 patients) by comparing the distance and the colour of the complex produced by standard solutions with the distance and colour of 20 predicted amino acids. The eight amino acids were identified in urine samples in this study (glutamine, threonine, asparagine, leucine, serine, alanine, glycine). The comparative biostatistical analysis between groups of patients and healthy people in the study showed the following results: (i) the level of glutamine in urine for patients with nephrotic syndrome more than in the healthy children, where the percentage in the infected was 22%, while in the healthy ones was 5%, and the probability value was $p = 0.0001$. (ii) There are significant differences in the level of asparagine between patients and healthy, where the value of the amino acid for patients was 38%, while in the healthy controls it was recorded 46% with a significant difference at the level of probability ($P<0.001$), (iii) A significant decrease of serine in the urine of patients, and it was 3% compared with the healthy 22%, with $p = 0.0001$, (v) Decrease in the percentage of lysine in the urine of patients with nephrotic syndrome16% compared to the healthy 46% with a level of probability ($P<0.0001$), these results clearly indicate to the relationship of the levels of various amino acids between the children with nephrotic syndrome and healthy children.

Keywords: Nephrotic Syndrome, Amino Acids, TLC chromatography.

Introduction

Nephrotic syndrome is a condition in which the kidneys infiltration of large amounts of protein into the urine (1). This can be considered a huge problem, which may lead to complicated disorders including swelling of body tissues and a higher chance of infection. Nephrotic syndrome is one of the most common reasons for referral to a pediatric nephrologist for evaluation, although its malignant appearance often delays diagnosis (2). Disorders of protein and amino acid metabolism can include conditions that occur when some amino acids cannot be broken down or produced by the body, resulting in a toxic build-up of some substances and/or a deficiency in others, although the nephrotic syndrome can affect people in any age,it is usually first diagnosed in children between the ages of 2 and 5 years (1).

Prior studies were conducted for several centres in the United States of America for children and adults showed that the most important causes of nephrotic syndrome (NS) are: Minimal Change Disease (MCD 27%), Focal segmental glomerulosclerosis (FSGS32%), membranous nephropathy 15% and other glomerular nephropathy 27% (3).An American study showed thatthe higher incidence of African American children compared to children of European descent (4). This study showed that African American children have a higher risk to develop FSGS compared to children of European origin and in general they are more likely to develop nephrotic syndrome than European children and this was demonstrated by kidney biopsy 42-72%. The probability of having Steroid-resistant nephrotic syndrome (SRNS) alsovaries by both the ethnic and geographic location, with 20% of cases reported in European children, 16-27 % in Africans, 27-54% in Asians and 20-39% in South Asians (5).

In Iraq, a recent study showed that there is a relationship between blood pressure and nephrotic syndrome, and the high blood pressure in patients with nephrotic syndrome in males more than in females (6). Therefore, nephrotic syndrome (NS) is currently considered a common disease in children where the incidence of NS in childhood has been reported as 4.7 (range 1.15-16.9) per 100,000 children worldwide, with great variation according to ethnic differences and geographical location (7). In addition, it is diagnosed by observing the leakage of protein from the blood into the urine through the damaged glomeruli. In hypoalbuminemia (albumin <25 g/L) and edema, the percentage of protein in urine increases (hourly ≥40 mg/m^2) or the percentage of protein/creatinine in urine 200 mg/mL or by the appearance ofprotein in a special strip to measure protein in the urine.Many cases of nephrotic syndrome were recorded, where they were diagnosed as hereditary.As a result, the appearance of pathological symptoms during the first three months of the child's life, and these children usually have a genetic mutation that affects either the endothelial cell or the glomerular basement membrane. Gene mutations in the structure and function of podocytes lead to kidney dysfunction, often present in either birth or steroid-resistant nephrotic syndrome (8). Some of the early genetic disorders were diagnosed included genes that signal the formation of the Notch barrier proteins nephrin (NPHS1) and podocin. (NPHS2) (9).

This work focused on the identification of the amino acids in which a disorder will occur due to nephrotic syndrome, and which influence amino acid metabolism to provide evidence of the relationship between the disorder of amino acid metabolism with nephrotic syndrome in children. In addition, thin layer chromatography was used as a technique for separating different amino acids. The results of this workmay help in finding appropriate treatments and diet for patients with nephrotic syndrome.

Research Design and Methods

Design Study

This work was performed at the Department of Chemistry, College of Science, University of Kerbala, Karbala, Iraq, and in cooperation with Karbala Teaching Hospital for Children, Karbala, Iraq. During the period of workfrom February 2020 to March 2021,the study involved a hundred participants (mean age ± SD: 7.25 ± 1.4 years). Prior to participation in this study, all the families of the participants were informed about the study to obtain their consent to participate their children in this study, with keeping the personal privacy of each case.

Collecting Urine Samples

The samples of urine were collected in the amount of 5 ml of urine from patients and healthy children of 100 samples in a normal tube and then they were placed in a centrifuge for 10 minutes at a speed of 4000g/per minute for the purpose of obtaining a precipitate for the purpose of exploring the components present, as well as checking the protein and examining the general urine, then withdrawing Part of the urine after completion of the sedimentation process by the pipet to store it at a temperature of 4 ° C by freezing for the purpose of conducting TLC chromatography.

Determination of Retardation Factor (Rf) for Amino Acids

The solutions of 20 standard amino acids were prepared by dissolving 1 mg in 1 ml of each amino acid in a 0.01 molar phosphate buffer solution (PH = 8.0). The one spot of the standard was placed on the thin

chromatography layer, and after that allow to dry at lab temperature. The thin paperwas placed in the container designated for the work that contains 70% propanol with 30% distilled water until the solvent reaches the distance, which was about 7 cm. After the solvent reaches the set limit, the paper was dried,and after drying the paper was sprayedby the solution of (6-Pyridin-2-yl-5,6-dihydro-benzo[4,5] imidazo[1,2c] quinazoline (0.01g/100ml),and then the TLC paper was dry at 90°C for 10 minutes. The TLC paper was sprayed with the solution of ninhydrin (0.01g/100ml), and the TLC paper was dried at 110°C for 10 minutes to form the unique colour for each standard amino acid as shown in Fig.1.

Figure 1. Describes the level of migration and colour contrast of standard amino acids.

The same methodology was applied to determine the amino acids in the urine samples of 100 participants (patients and healthy children) by measuring the distance Rf and checking the colour that appeared and comparing the result of each determined sample with the relevant standard amino acid.

Statistical Study

Graph Pad Prism version 8 “Graph Pad software, CA, USA” was used to analyse the data in this work. Due to the not numerical data, the Chi-square test (X^2) was applied to distinguish between the appearance and absents of the amino acid in the urine samples of the patients (P) and healthy volunteers (HV). The statistical significance was considered at $P < 0.05$.

Results and Discussion

Thin-layer chromatography (TLC) was used to examine 100 urine samples for each of 50 children with nephrotic syndrome, compared to 50 samples for healthy children. By comparing the distance of amino acids movement (Rf) was determined, as well as the distinctive colour with the standard solutions of amino acids. Consequently, the identity of the amino acid appearing in the adrenal was determined. Depending on the results, a biostatistical analysis was conducted and a comparison between the levels of amino acid appearance or not for the examined samples (patients and healthy volunteers), Fig. 2.

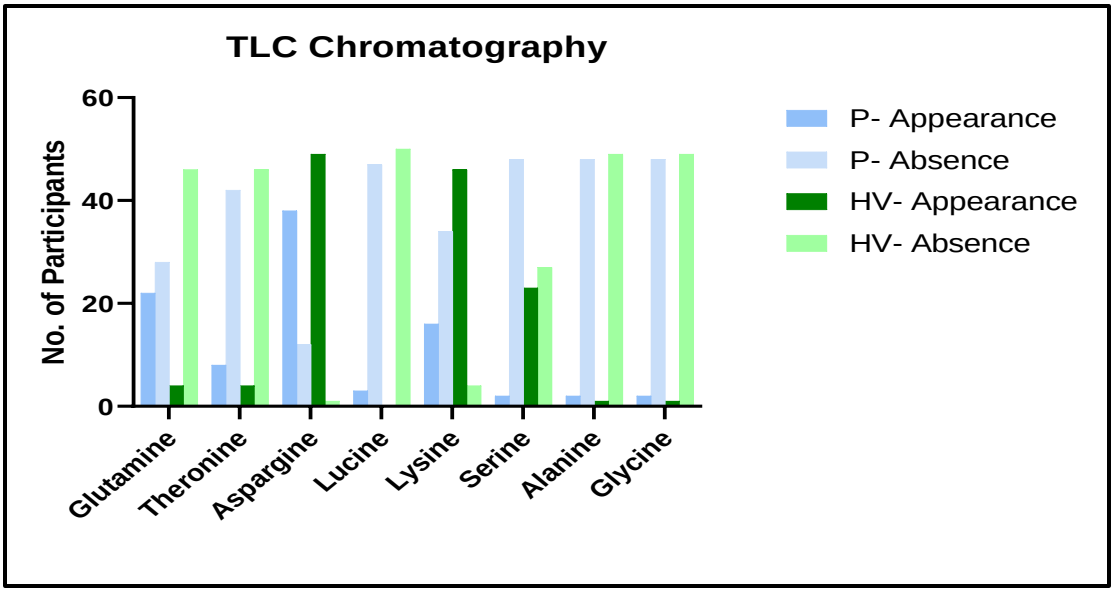

Figure 2. Determination of amino acids by TLC technique (TLC) between patients with nephrotic syndrome (P) and healthy subjects (HV).

The current study indicates the presence of the amino acid glutamine in the urine of patients with nephrotic syndrome more than the healthy ones, where the percentage of those with nephrotic syndrome was 22%, while in the healthy ones it was 5%, and the value of chi-square was (16.84) and the p-value = 0.0001 which supports the existence of a fundamental difference as shown in Fig.3.

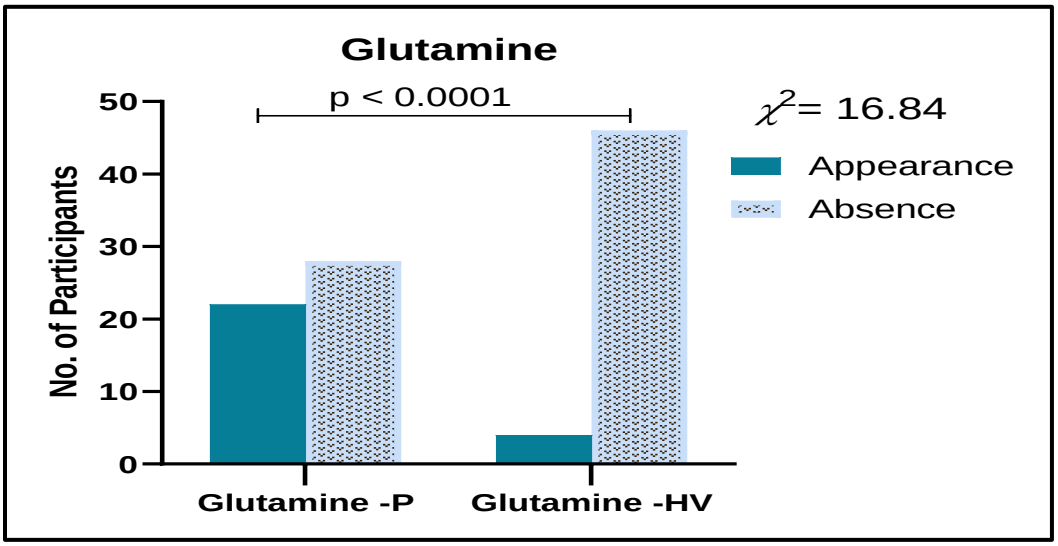

Figure 3. The statistical analysis of the determination of glutamine in urine.

One of the reasons for excretion of the amino acid glutamine from the kidneys in healthy people is due to the metabolism of acidosis (metabolic acidosis). During this process, ammonium ions are excreted through the urine, as well as include the secretion of amino acids and bicarbonate ions to compensate for the acidity of the blood (10). Glutamine is the most important NH_3-donor amino acid and plays an important role in the kidney function. It may be considered as an indicator of kidney disease, and it is

primary importance in intermediate metabolism and the exchange of nitrogen between organs through the transport of ammonia (NH_3) between tissues to maintain pH (11). A significant increase ($P < 0.05$) was observed in the value of glutamine in patients with urine, which was recorded at 22% compared to the healthy group, where it was recorded at 5%. Chronic kidney disease, which was higher in glutamine and glycine than the healthy group (12), also differed with the study, which showed that acute and chronic renal failure are not accompanied by any high concentrations of glutamine acid in the plasma, and he attributed these reasons to poor cellular integrity, in general it is acid. Some studies have shown that glutamine plays a vital role in the metabolism of glutathione, in addition to that it maintains cellular oxidation during elevated cellular oxidative stress is essential for alleviating stress-induced oxidative disorders in diabetes (13-15).

The statistical analysis of the data of thyronine has showed that there are no significant differences between patients and healthy children, as it scored chi-square (1.515) with no significant differences ($P = 0.218$) as shown in Fig.4.

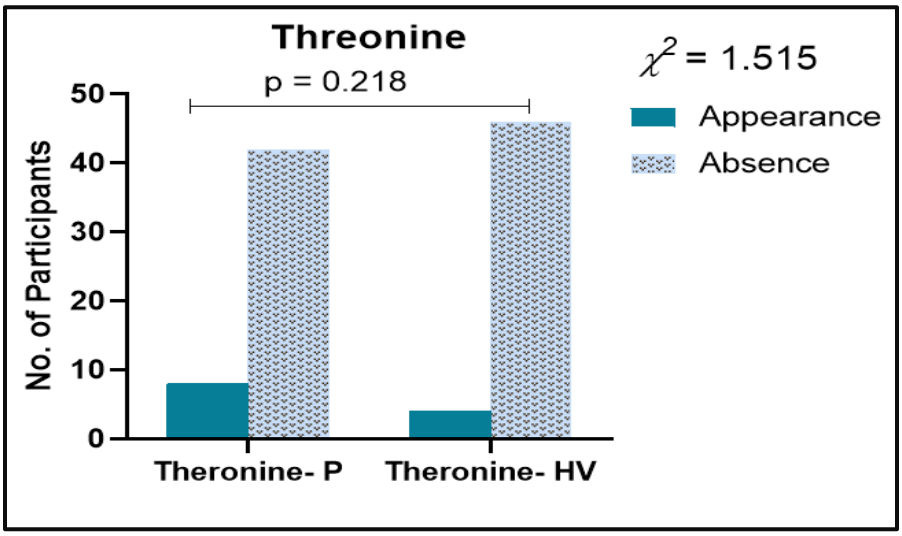

Figure 4. The statistical analysis of the determination of threonine in urine.

The results of the determination of asparagine have appeared that there are significant differences in the level of asparagine between patients and healthy controls, where the value of the asparagine for patients was 38%, while in the healthy control it was recorded 46% with a significant difference with a value of chi-square (10.70) and a level of probability ($P < 0.001$), as shown in Fig.5.

Some genesrelated to the actin cytoskeleton, transcription factors, nucleus, glomerular basement membrane, mitochondria, and other proteins can affect cell podocytes. The As paragine-linked glycosylation gene plays a major role in the pathogenesis of the nephrotic syndrome(16).

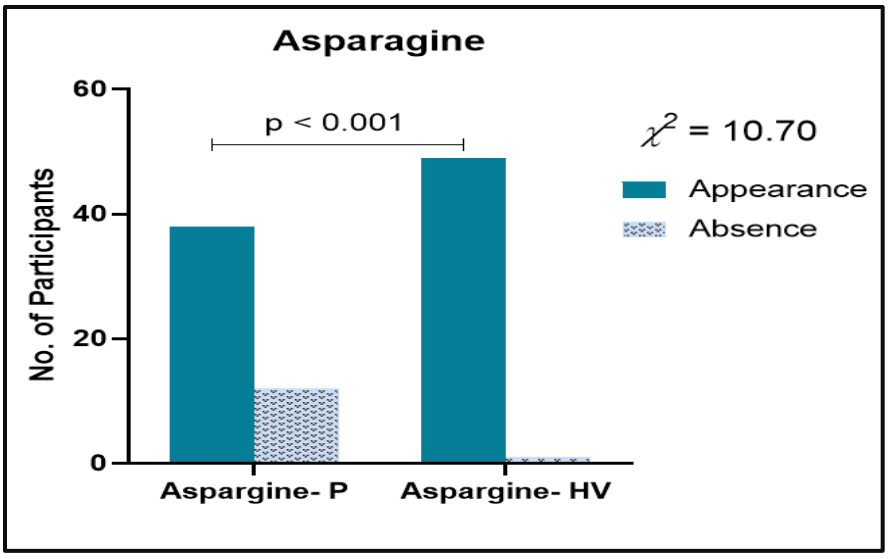

Figure 5. The statistical analysis of the determination of as paragine in urine.

The statistical analysis showed that there is a significant decrease in serine in the urine of patients, which was 3%, compared with the healthy volunteers, which was 22%, Fig.6.Due to the serine has cellular toxicity, so the high level has a clear role in chronic kidney disease, and the exacerbation of cellular imbalance caused by the serine, which can lead to the death of tubular cells or their gradual damage (17). Some studies also revealed that the accumulation of serine in the blood plasma is linked to faster development in chronic kidney patients (18). A previous report revealed that the levels of serine were elevated in elderly individuals and patients with chronic kidney disease. However, other studies suggested that the risk of progression to the end stage of renal disease was approximately three times higher in chronic kidney patients, who had the highest levels of serine in plasma (19).

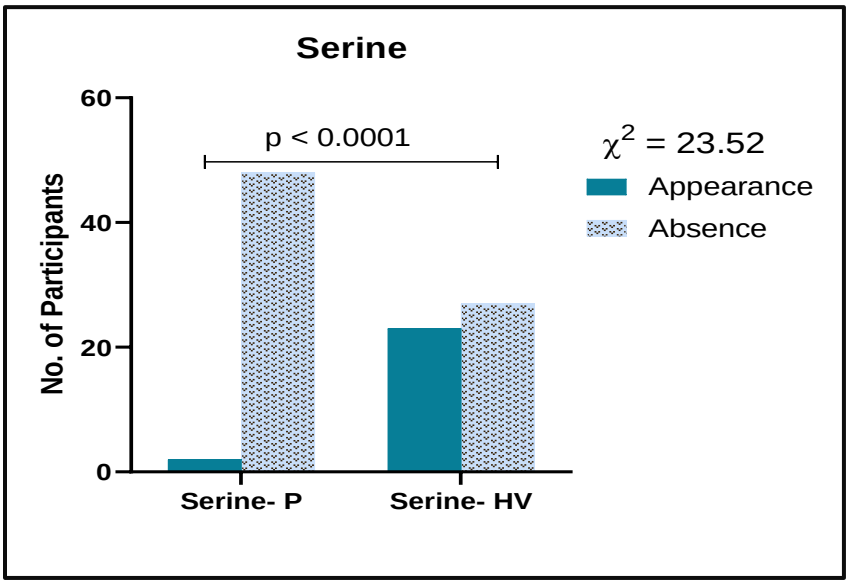

Figure 6. The statistical analysis of the determination of serine in urine.

The results have shown that the level of thelysine was low in the urine of patients 16% compared to the healthy volunteers 46%, as shown in Fig.7, with the value of chi-square (38.20) and probability (P<0.0001). Some studies have shown that a high dose of lysine is able to cause acute renal failure (20). The kidneys are sensitive to metabolic changes and metabolic phenotyping revealed several pathways associated with kidney disease, and the kidneys play an essential role in the lysine cycle (21).

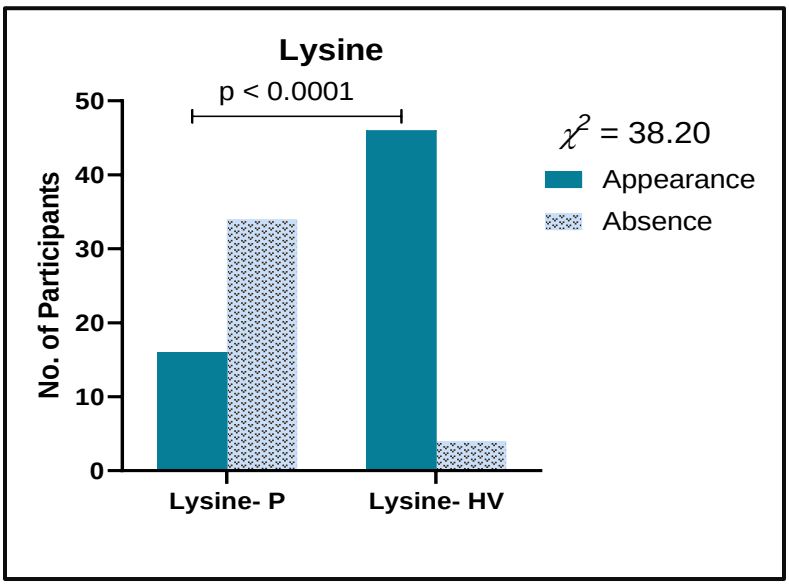

Figure 7. The statistical analysis of the determination of lysine in urine.

Conclusions

Nephrotic syndrome is one of the common pathological cases in children within the age group (2-10 years), especially in the specific study area in Karbala, Iraq.The identification of amino acids in urine samples that were conducted in the study (50 patients and 50 healthy children) by comparing the migration distance and the colour of the complex produced in migration with samples of standard solutions for 20 potential amino acids by using the TLC technique.

A qualitative diagnosis was performed for eight amino acids that appeared in urine samples of the studied models, which are (glutamine, threonine, asparagine, leucine, serine, alanine, glycine). The comparative statistical diagnosis between groups of patients and healthy volunteers in the study showed the following results: (A) the glutamine in the urine of patients with nephrotic syndrome more than in the healthy, where the percentage in the infected was 22%, while in the healthy it was 5% and the probability value was p = 0.0001, (B) appearance of the threonine in the urine of healthy and patients with no significant differences (P = 0.218), (C) There are significant differences in the level of as paragine between patients and healthy subjects, where the value of the amino acid for patients was 38%, while in the healthy controls it was recorded 46% with a significant difference at the level of probability (P<0.001), (D) A significant decrease in The percentage of the serine in the urine of patients which was 3% compared to the healthy children, which was 22% with the level of probability p = 0.0001, (E) recording a decrease in the percentage of thelysine in the urine of patients with nephrotic syndrome (16%) compared to the healthy volunteers, it was (46%)with the level of probability (P<0.0001).These

results clearly indicate the existence of a relationship between the difference in the level of various amino acids in the studied samples between children with nephrotic syndrome and healthy children.

This work highlighted the importance of using thin-layer chromatography (TLC) to identity of amino acids in the urine of children with nephrotic syndrome throughout the grate obtained results. Thus, avoiding the pathological complications that may result from the development of the pathological condition of children with nephrotic syndrome, by utilizing the appropriate treatment. In addition, it can be regulated the nutrition of the children with nephrotic syndrome or provided them with important nutritional supplements to compensate for the deficiency in some amino acids resulting from the metabolic disorders associated with the disease.

Acknowledgements

The authors would like to thank the department of chemistry, college of science, university of kerbala for all support.

Funding Sources

The authors received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors declare no conflict of interest.

References

1. A. Pal and F. Kaskel, History of Nephrotic Syndrome and Evolution of its Treatment. Frontiers in Pediatrics, **4**, 1-6 (2016).https://doi.org/10.3389/fped.2016.00056.
2. D. S. Gipson, J. P. Troost, R. A. Lafayette, M. A. Hladunewich, H. Trachtman, C. A. Gadegbeku, J. R. Sedor, L. B. Holzman, M. M. Moxey-Mims and K. Perumal, Clinical Journal of the American Society of Nephrology, 11, 81-89 (2016).https://doi.org/10.2215/CJN.02560315.
3. M. L. Downie, C. Gallibois, R. S. Parekh and D. G. Noone, Nephrotic syndrome in infants and children: pathophysiology and management. Paediatrics and International Child Health, **37**, 248-258(2017).https://www.tandfonline.com/doi/abs/10.1080/20469047.2017.1374003?journalCode=ypch20.
4. E. Klebs, "Handbuch der pathologischen Anatomie", (Verlag von August Hirschwald, Berlin, German, 1870) pp.325-340.https://archive.org/details/b28056863/page/ 24/mode/2up.
5. J. S. Cameron, The Nephrotic Syndrome and Its Complications. Am J Kidney Dis., **10**(3), 157-171 (1988).https://linkinghub.elsevier.com/retrieve/pii/S0272638687801701.
6. S. S.Jassem and A. R. R. Mohammed, Determination of total protein in human blood by laser. Diyala Journal for Pure Science, **13**(3), 262-275 (2017).https://djps.uodiyala.edu.iq/uploads/Volume%2013/Issue%203/part%202/20e-p2(PEB2).pdf.
7. D. H.Golwyn Jr, Routh, W. D., Chen, M. Y., Lorentz, W. B. and, R. B.Dyer, Percutaneous transcatheter renal ablation with absolute ethanol for uncontrolled hypertension or nephrotic

syndrome: results in 11 patients with end-stage renal disease. Journal of Vascular and Interventional Radiology, **8**(4), 527-533 (1997).https://www.jvir.org/article/S1051-0443(97)70604-3/pdf.

8. H. S. Schutta, Richard Bright's observations on diseases of the nervous system due to inflammation. Journal of the History of the Neurosciences, **27**(2), 165-185 (2018).https://www.tandfonline.com/doi/full/10.1080/0964704X.2017.1382804.
9. R. P. Hull and D. J. A. Goldsmith, Nephrotic syndrome in adults. BMJ.**336**, 1185-1189 (2008).https://www.bmj.com/content/336/7654/1185.
10. J. A.Kraut and N. E. Madias, Metabolic acidosis of CKD: an update. American Journal of Kidney Diseases, **67**(2), 307-317(2016).https://doi.org/10.1053/j.ajkd.2015.08.028.
11. I. D.Weiner and J. W.Verlander, Renal Ammonia Metabolism and Transport. Compr. Physiol. **3**, 201-220 (2013).https://onlinelibrary.wiley.com/doi/10.1002/cphy.c120010.
12. A. Roointan, Y. Gheisari, K. L. Hudkins and A. Gholaminejad, Non-invasive metabolic biomarkers for early diagnosis of diabetic nephropathy: Meta-analysis of profiling metabolomics studies. Nutrition, Metabolism and Cardiovascular Diseases. 31(8), 2253-2272 (2021). https://www.nmcd-journal.com/article/S0939-4753(21)00214-3/fulltext.
13. V. M. Abdulrazzaq, T. M. M. Al-Rammahi and A. W. Ashor, Association of hand grip strength with cardiometabolic markers in type 2 diabetes mellitus. AIP Proceedings Conference. **2290**(030041), 1- 9 (2020).https://doi.org/10.1063/5.0027494.
14. M. Kodama and K. I. Nakayama, A second Warburg-like effect in cancer metabolism: The metabolic shift of glutamine-derived nitrogen: A shift in glutamine-derived nitrogen metabolism from glutaminolysis to de novo nucleotide biosynthesis contributes to malignant evolution of cancer. BioEssays,**42**(12), 2000169 (2020).https://doi.org/10.1002/bies.202000169.
15. T. M. M. Al-Rammahi, H. Al-Rubaye and A. W. Ashor, The Association of Higher Composite Biomarker Score of Antioxidant Vitamins with Lower Cardiovascular Diseases Risk: A Cross-Sectional Study. Curr Res Nutr Food Sci.**9**(3), 945-952 (2021).http://dx.doi.org/10.12944/CRNFSJ.9.3.21.
16. C. Kranz, J. Denecke, L. Lehle, K. Sohlbach, et. al., Congenital disorder of glycosylation type Ik (CDG-Ik): a defect of mannosyltransferase I. The American Journal of Human Genetics, 74(3), 545-551(2004).https://doi.org/10.1086/382493.
17. D. Darmaun, L. Torres-Santiago and N.Mauras, Glutamine and type 1 diabetes mellitus: is there a role in glycemic control?Current Opinion in Clinical Nutrition & Metabolic Care, 22(1), 91-95(2019).https://pubmed.ncbi.nlm.nih.gov/30461450/.
18. S.-L. Yeh, Y.-M. Shih and M.-T. Lin,Chapter 25: Glutamine and its antioxidative potentials in diabetes. In: Preedy VR, editor. Diabetes (Second Edition): (Academic Press; 2020), p.p 255-264.https://doi.org/10.1016/B978-0-12-815776-3.00025-5.
19. M. Suzuki, Y. Gonda, M. Yamada, et al., Serum D-serine accumulation after proximal renal tubular damage involves neutral amino acid transporter Asc-1. Sci Rep**9**, 1-11 (2019). https://doi.org/10.1038/s41598-019-53302-2.

20. T. Kimura, A. Hesaka and Y. Isaka, Utility of d-serine monitoring in kidney disease. Biochim Biophys Acta Proteins Proteom. **1868**(9), 140449 (2020).https://doi.org/10.1016/j.bbapap.2020.140449.
21. N. Rabbani and P. J. Thornalley, Advanced glycation end products in the pathogenesis of chronic kidney disease. Kidney international, 93(4), 803-813(2018).https://doi.org/10.1016/j.kint.2017.11.034.
22. Jalil, A. T., Al-Khafaji, A. H. D., Karevskiy, A., Dilfy, S. H., & Hanan, Z. K. (2021). Polymerase chain reaction technique for molecular detection of HPV16 infections among women with cervical cancer in Dhi-Qar Province. Materials Today: Proceedings. https://doi.org/10.1016/j.matpr.2021.05.211
23. Hanan, Z. K., Saleh, M. B., Mezal, E. H., & Jalil, A. T. (2021). Detection of human genetic variation in VAC14 gene by ARMA-PCR technique and relation with typhoid fever infection in patients with gallbladder diseases in Thi-Qar province/Iraq. Materials Today: Proceedings. https://doi.org/10.1016/j.matpr.2021.05.236
24. Jalil, A. T., & Karevskiy, A. (2020). The Cervical Cancer (CC) Epidemiology and Human Papillomavirus (HPV) in the Middle East. International Journal of Environment, Engineering & Education, 2(2), 7-12. https://doi.org/10.5281/zenodo.3972634
25. Turki Jalil, A., Hussain Dilfy, S., Oudah Meza, S., Aravindhan, S., M Kadhim, M., & M Aljeboree, A. (2021). CuO/ZrO2 nanocomposites: facile synthesis, characterization and photocatalytic degradation of tetracycline antibiotic. Journal of Nanostructures.
26. Jalil, A. T. (2020). COVID-19 most affected age groups and lethality in Europe, Glob. J. Public Health Med, 2, 179-184. https://doi.org/10.37557/gjphm.v2iSP1.51
27. Mezal, E. H., Yousif, A. F., Hanan, Z. K., Hanan, A. K., & Jalil, A. (2020). Isolation, Assessment of Antimicrobial Sensitivity of Bacterial Pathogens from Post-Cesarean section Infection of patients in Thi-Qar Province. European Journal of Molecular & Clinical Medicine, 7(3), 958-964.
28. Mubark, N. N., Jalil, A. T., & Dilfi, S. H. (2020). DESCRIPTIVE STUDY OF HYDATIDIFORM MOLE ACCORDING TO TYPE AND AGE AMONG PATIENTS IN WASIT PROVINCE, IRAQ. Global Journal of Public Health Medicine, 2(1), 118-124. https://doi.org/10.37557/gjphm.v2i1.30
29. Turki Jalil, A. ., Dilfi, S. H. ., & Karevskiy, A. . (2019). SURVEY OF BREAST CANCER IN WASIT PROVINCE , IRAQ. Global Journal of Public Health Medicine, 1(2), 33–38. https://doi.org/10.37557/gjphm.v1i2.7
30. Jaleel, A. T. (2018). SURVEY THE PREVALENCE OF VIRAL HEPATITIS A, B, C INFECTION IN DHI-QAR PROVINCE (IRAQ). ББК 20.1 А43 Редакционная коллегия: ИБ Заводник (отв. ред.), АЕ Каревский, ОВ Янчуревич, ОВ Павлова, 95.
31. Jalil, A. A. T. EPIDEMIOLOGY OF CERVICAL CANCER AND HIGH RISK OF HUMAN PAPILLOMA VIRUS IN PATIENT. ББК 28.6 3, 85(7).
32. Roomi, A. B., Widjaja, G., Savitri, D., Turki Jalil, A., Fakri Mustafa, Y., Thangavelu, L., ... & Aravindhan, S. (2021). SnO2: Au/Carbon Quantum Dots Nanocomposites: Synthesis, Characterization, and Antibacterial Activity. Journal of Nanostructures.

33. Raya, I., Chupradit, S., Mustafa, Y., H. Oudaha, K., M. Kadhim, M., Turki Jalil, A., J. Kadhim, A., Mahmudiono, T., Thangavelu, L. (2021). Carboxymethyl Chitosan Nano-Fibers for Controlled Releasing 5-Fluorouracil Anticancer Drug. Journal of Nanostructures