Prevalence of Thyroid Dysfunction in Children Visiting OPDs of Different Hospitals of Twin Cities

SADAF SULTANA¹†, MUHAMMAD BILAL^{1,2}*, FEROZA HAMID WATTOO¹, ASMA NAFISA³, SYEDA SAMAN BATOOL¹, MARYAM SADAQAT¹, AHMAD FARAZ¹

¹University Institute of Biochemistry and Biotechnology, PMAS Arid Agriculture University Rawalpindi, Pakistan ²School of Life Sciences and Medicine, University of Science and Technology of China, China ³Department of Clinical Studies, Rawalpindi Medical University, Pakistan

Abstract- The thyroid gland is the most crucial factor in regulation of body metabolism, neurological development and growth in children. Any abnormality that results in change of thyroid secretion causes thyroid disorders. These changes could be either functional or structural. Congenital and acquired thyroid dysfunctions are among the most frequent pediatric endocrine disorders, yet their recognition remains insufficient in lowresource settings. Pakistan's twin cities lack pediatric thyroid epidemiology data. Our study aimed to addresses following objectives by finding frequency of thyroid disorders among 1-12 years of pediatrics population, identifying categories of thyroid disorders in prospective patients and evaluating influence of nutritional risk factors encountered in thyroid dysfunction patient. A total 215 OPD patients visiting the hospitals over a period of eight months (August 2022 to March 2023) were screened. Children between ages of 1-12 years who visited the children OPD of different medical centers in twin cities were counted in if they had clinical impression of thyroid disorders. All of the 215 children were examined for thyroid profile comprising of Triiodothyronine (T3) serum level, Thyroxin (T4) level and Thyroid stimulating hormone (TSH) serum levels. For the purpose of statistical determination and analysis, SPSS software version 25 was used to estimate prevalence rate of thyroid dysfunctions. Overall, our study our found that subclinical and overt hypothyroidism were more prevalent (11.2%) thyroid dysfunction as compare to other types in the children Prevalence of thyroid dysfunction was indicated greater in school-aged children and females. Nutritional disorderness caused due to deficiency of essential micronutrients (iodine, selenium, iron) as well as a diet high in goitrogen could be a considerable contributing factor in initiation of thyroid dysfunction. Often clinical demonstrations are non-specific (e.g., short stature, constipation), indicating the need of proper diagnostic test instead of relying on usual signs like goiter.

Index Terms- Endocrine, Goitrogen, Micronutrients, Pediatric, Thyroid disorder, Thyroxin, Triiodothyronine

I. INTRODUCTION

he thyroid gland is the most crucial factor in regulation of body metabolism, neurological development and growth in children. Any abnormality that results in change of thyroid secretion causes thyroid disorders. These changes could be either functional or structural [1]. Three different causes could be found for the onset of thyroid disorders. Primary is disruption of gland,

secondary is due to pituitary illness and tertiary is due to damage of hypothalamus. Children frequently suffer from this disorder [2]. Globally, thyroid dysfunction in children ranges from 2% to 15%, varying by geographic region, iodine intake, and diagnostic criteria. In South Asia, particularly Pakistan, the burden of undiagnosed pediatric thyroid disorders is aggravated by a lack of routine screening programs, insufficient awareness among parents and healthcare providers, and socioeconomic barriers to healthcare access. Despite these challenges, limited data exist on the prevalence of thyroid dysfunction in pediatric population of Pakistan, especially in urban centers like Rawalpindi and Islamabad. [3]. Severity of this disorder varies and is influenced by different variables including gender, age, location etc. [4]. Thyroid gland is situated just at anterior position of neck and is vascular organ having weight of 15-20 grams. Men have heavier thyroid gland as compared to women. Newborns have thyroid gland of weight 1 gram and it increases until age of 15 years at rate of 1 gram per year [5]. Thyroid gland had almost shape similar to letter H, it is a red soft organ comprising of mainly two muscular lobes connected by isthmus one on each side, one left and one right and is reddish in color [5, 6]. Thyroid stimulating hormone (TSH) is also termed as thyrotropin. Mainly TSH is released by the anterior pituitary gland and further enhance the production and secretion of thyroid hormones. TSH further trigger thyroid gland to secrete thyroxine (T4) and 3, 5, 3'-triiodothyronine (T3) [7, 8].

Figure 3: Structure of thyroid hormones [9]

Thyroid malfunction is difficult to diagnose on basis of clinical features of patient because of broad-spectrum of overlapping clinical features with other diseases and exceptionally malleable manifestations. So, biochemical methods are highly recommended for proper prognosis of thyroidal dysfunctions. Thyroid Stimulating hormone (TSH) system stimulates the normal functioning of thyroid and any sort of change in thyroid hormones like 3,5,3'-triiodothyronine (T3), Thyroxine (T4) and decrease in thyroid stimulating hormone serum exerts severe case of thyroid dysfunction. Likewise, thyroid stimulating hormonal profiling or biomedical testing are recommended for proper indication of

thyroid disorders. The serum profiling of T3 and T4 are performed in case of hyper volume of TSH in contrast with normal reference rages of TSH [10].

Primarily TSH serum level is measured because the molecular level changes firstly occur in TSH secretion and then in T3 and T4 hormone by altering their concentrations level. The level of TSH is recommended to be analyzed and investigated by measuring volume concentrations of TSH when fall among 0.4 milli units per litre (mU/L) and 4.5 mU/L. Although, investigation of Thyroid Stimulating Hormone is often deliberated as first line testing even it is much more persistent and reliable than fluctuating concentration levels of plasma T3 and T4. Negatively inhibition of anterior thyroid reveals the TSH levels in primary Hypothyroidism [11].

Thyroid dysfunction, in case of hypothyroidism hyperthyroidism, can affect badly on child's mental and physical development. In pediatric population most prevalent endocrine diseases are congenital and acquired thyroid disorders, yet they are difficult to recognize in recognition low-resource settings. Pakistan's twin cities, Rawalpindi and Islamabad still possess less pediatric thyroid epidemiology data [12, 13]. This core intention of research was to link this knowledge gap by conducting a broad prevalence study across OPDs of the different hospitals in the twin cities. It is important to know disease patterns and its early detection and management therefore our study aimed to addresses this by finding frequency of thyroid disorders among 1-12 years of pediatrics population, identifying categories of thyroid disorders in diagnosed children and evaluating nutritional risk factors effecting thyroid dysfunction patient.

II. METHODOLOGY

Children between ages of 1-12 years who visited the Pediatric OPDs of different medical centers in twin cities were counted in if they displayed clinical signs of thyroid disorders. Institute Ethics Committee granted ethical approval and a parent or legal guardian of each patient provided a written informed consent. Our research was a clinical based study which was performed to evaluate the prevalence of thyroid disorders among pediatric population. A total 215 OPD patients visiting the hospitals over a period of eight months (August 2022 to March 2023) were examined. Children of 1 to 12 years of age with clinical symptoms indicative of thyroid dysfunctions such as persistent jaundice in neonatal, goiter, symptoms of cretinism, global development delay, obesity and constipation were included in study. Suspected children who were taking medicine that could possibly change the thyroid hormone status and patient in whom parents were reluctant to provide informed consent and their clinical history were not consider for the research. Anthropometric data were collected and assessed against the CDC reference values, vital considerations were assessed and comprehensive physical investigation was done. Assessment of the functioning of thyroid gland was performed as per WHO guidelines [14]. A questionnaire was designed to evaluate pediatric age, sexual characteristics, height, weight and a nutritive dietary feedback, which included the entities' diet history as well as their presented sign and symptoms (e.g. cold intolerance, short stature, goiter, anxiety, and constipation). Dietary history intakes were computed and examined [15].

All of the 215 children were examined for thyroid function test

consisting of T3, T4 and TSH serum level measurement. In a plain vial 5 ml blood sample was collected via venipuncture under aseptic conditions in the early morning after intake of light breakfast the laboratories of the hospitals. Following the clotting period of 20 minutes, the sample was centrifuged at 2000 rev for about 25 minutes for separation of serum from other components of blood. The separated serum aliquots were labelled and stored at -200C, until assays were performed. Immulite Special Chemistry Analyzer based on enzyme chemiluminescence assay working similar to the principle of Enzyme Labelled Immunosorbent Assay (ELISA) was used to diagnose thyroid dysfunction. Different identification test including TSH, T3, T4, FT3, FT4, and thyroglobulin, Screening Test for analysis of thyroid dysfunction were performed.

The frequency estimation was done by execution of different biochemical identification Test including T4, Free T4, TSH, Free T3 and T3. Subsequently biochemical assessment of thyroid dysfunction disorders was classified as Overt hypothyroidism, subclinical hypothyroidism, normal, subclinical hyperthyroidism and hyperthyroidism case depending on the basis of level of concentration of serum T4, TSH and T3 hormone with respect to their particular standard cut off values. Categories of various thyroid dysfunctions are illustrated in Table 1.

Table 1: Categories of thyroid dysfunctions

Category of	Levels of Thyroid Hormone in blood serum			
Disorder	TSH	T4	Т3	
Hypothyroidism	Greater than standard range	Less than standard range	Less than standard range	
Subclinical Hypothyroidism	Greater than standard range	Equal to standard value	Equal to standard value	
Hyperthyroidism	Less than standard range	Greater than standard range	Greater than standard range	
Subclinical Hyperthyroidism	Less than standard range	Equal to standard value	Equal to standard value	

Statistical evaluation and analysis

For the purpose of statistical determination and analysis, SPSS software version 25 was used to find out prevalence of thyroid dysfunction. Various parameters of descriptive stats were applied including frequency analysis, Mean determination were executed for the analysis of design questionnaire. These all parameters were used to find out status of thyroid dysfunction in pediatric population of Rawalpindi and Islamabad.

III. RESULTS

A total of 215 children were screened from which 88 belongs to 7-12 years age group, among which 34 (38.6%) were dysfunction cases. Out of 71 toddlers (1-3 years), screened 23 were diagnosed with thyroid dysfunction having the prevalence rate of 32.3%, Table 1 indicates that approximately one-quarter of preschooler (4-6 years) group had thyroid dysfunction. On the whole screened male vs female ratio was 1:1.3 while diagnosed female cases with thyroid dysfunction was found to be 46 (37.7%) in contrast to this male which were 24 with prevalence rate of 25.8% suggesting higher rate prevalence of thyroid dysfunction in females. [Table 2]

Table 2. Frequency estimation of thyroid disorders based on

gender and age

Charact	eristics	Total screened	Dysfunction cases (N)	Prevalence (%)
Age	1-3	71	23	32.3
(years)	(Toddlers)			
	4-6	56	13	23.2
	(Preschooler)			
	7-12	88	34	38.6
	(School age)			
Gender	Males	93	24	25.8
	Females	122	46	37.7

Among 215 examined children, 70 were diagnosed with thyroid dysfunction, among those 24 children were diagnosed with hypothyroidism (11.1%) and 3 with hyperthyroidism (1.4%) while 32 (14.8%) were having subclinical hypothyroidism and 11 belongs to subclinical hyperthyroid cases with the prevalence of (5%) [Figure 2].

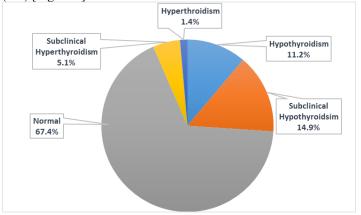


Figure 2: Identification of prevalence of type of thyroid Dysfunction.

It was found that only 11.4% children diagnosed with thyroid dysfunction were consuming iodine rich foods, while a larger percentage (81.4%) of dysfunctional children were involved in consuming goitrogen containing foods a major reason for disruption in synthesis of thyroid hormone. [Table 3].

Table 3: Evaluation and Comparison of intake of various dietary foods among children with thyroid dysfunction

Nutritional	Frequency (N) of	Percentage
intakes	children	
Selenium rich food	30	42.8%
Goitrogen intake	57	81.4%
Iron rich food	29	41.4%
Iodine rich food	8	11.4%

Figure 4 presents analysis of clinical manifestations associated with hyperthyroidism and hypothyroidism. Out of 24 hypothyroidism cases 12 (50%) exhibit cold intolerance indicating that a significant proportion of individuals with hypothyroidism a sensitive to cold temperature comparing to hyperthyroidism cases where heat intolerance is reported. In hypothyroidism, 6 tested children had positive family records of thyroid dysfunctions (25%) as compared to hyperthyroid cases having one children with

positive family history. Short stature (62%) was the most dominant emerged symptoms in children diagnosed with overt hypothyroidism followed by cold intolerance. Out of 24 hypothyroid children 14 (58%) were experiencing constipation indicating the prevalence of gastrointestinal symptoms in hypothyroidism cases. Overall, only 8.57 % of children diagnosed with thyroid dysregulation had goiter but it was mainly associated with 33% hyperthyroidism cases. Anxiety and weight loss were also major manifested clinical symptoms in hyperthyroidism case with prevalence of (100%) and (66%) The clinical manifestations of the patients with hypothyroidism and hyperthyroidism are listed in [Figure 3].

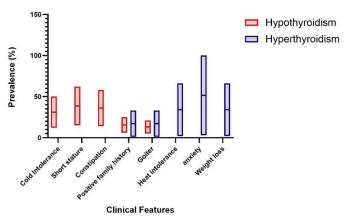


Figure 3: Evaluation and Comparison of the presented clinical features among suspected thyroid diagnosed patients.

IV. DISCUSSION

Thyroid glands play a vital role in body metabolism, in current research, we considered the thyroid profile of 215 suspected children to have thyroid dysfunction on clinical basis between age group 1-12 years. 70 cases of thyroid dysfunction were identified among overall screened 215 children with a total prevalence of 32.6%. The data indicates the significant prevalence of thyroid disorders as a pediatric health concern. Subclinical Hypothyroidism was found to be the most frequent abnormality with the prevalence rate of (14.8%), subsequently overt Hypothyroidism had a prevalence rate of (11.1%). The low prevalence of hyperthyroidism (1% overt, 5% subclinical) is accordance with global statistics showing that hypothyroidism is much more prevalent than hyperthyroidism in the general pediatric population [16].

Females exhibit a higher rate of prevalence (37.7%) of thyroid dysfunction as compared to males (25.8%) showed a clear gender disproportion. These results are in accordance with the well-known pattern in adult thyroidology, where Hashimoto's thyroiditis kind of autoimmune thyroid diseases are more common in female influenced by the hormonal and genetic factors [17].

Our study found that prevalence of thyroid dysfunction was much more in children of age 7-12 years (38.6%) indicating its association to the onset of autoimmune diseases. This finding aligns with the study by Pearce et al., (2003) which demonstrated that often autoimmune disease disorders are activated around this age and diagnostic symptoms are more apparent at this age as child began to mature. [18].

31-35

This study deeply analyzed that how dietary intakes are effecting prevalence of thyroid dysfunction. Intake of goitrogen was observed in large group of children (81.4%) diagnosed with thyroid dysfunction. Cooking usually deactivates most goitrogens but consuming a high amount of raw cruciferous vegetables could possibly aggravate thyroid dysfunction in the children already suffering with iodine deficiency or predisposition [19]. Our research found that intake of iodine-rich foods was significantly low (11.4%), in thyroid dysfunctional children which is an important concern. For Thyroid hormone production iodine is a crucial element and insufficient intake of iodine could be a serious cause of hypothyroidism and goiter in children worldwide [20]. Intake of selenium rich and iron-rich foods were found to be less in pediatrics population. Where in early studies selenium was founded to be a basic element required for formation of triiodothyronine (T3) from thyroxine (T4) furthermore it protects thyroid gland from oxidative damage [21]. Synthesis of thyroid hormone could be effected by iron deficiency as it leads to reduce activity of the enzyme thyroid peroxidase [22]. Thyroid dysfunction is often trigger by a compromised nutritional pattern where child is taking diet low in iodine, selenium, and iron and excessive goitrogen

In our study the evaluation of clinical features manifested in thyroid dysfunctional individuals demonstrate a comprehensive symptomology. Short stature (62%) and constipation (58%) were the most prevalent symptoms in the hypothyroid group, indicating effect of thyroid hormone deficiency on growth and gastrointestinal motility [23]. Cold intolerance (50%) was also common. The study shows that goiter (21%) or a positive family history (25%) were least prevalent signifying that depending solely on these signs is insufficient for diagnosis of accurate case. Evaluating the hyperthyroid cases anxiety was found to be diagnosed in all children (100%), followed by heat intolerance and weight loss were both present in 66% of cases. These align perfectly with the hyper adrenergic state caused by excess thyroid hormone [24].

Our study of pediatric thyroid dysfunction demonstrated a different clinical picture showing that symptoms often differ from typical expectations. During examination of When examining hypothyroid cases, it was found that most prevalent issues were short stature and chronic constipation, affecting well over half of the cohort indicating that low secretion of thyroid hormone could directly impact physical growth as well as gastrointestinal function. Cold intolerance was also most frequent symptom. Moreover, our study found that traditional indicators like goiter or a positive family history of thyroid dysfunction were far less common, in contrast to an early study reporting 38% prevalence of goiter in children diagnosed with thyroid hormone anomalies [13] indicating that a dependence on these signs only to accurate diagnosed that disease is not enough. Hyperthyroid cases revealed a markedly different situation. Anxiety was experienced by every child diagnosed with hyperthyroidism, emphasizing that thyroid hormone had great impact on development on the child nervous system. Children diagnosed with hyperthyroidism also suffer heat intolerance and unexplained weight loss. These findings align with early study by Sosnowska-Sienkiewicz et al., (2024) indicating onset of hyper-adrenergic state triggered by an overactive thyroid

Overall, our study our found that subclinical and overt

hypothyroidism were more prevalent thyroid dysfunction in the children, Prevalence of thyroid dysfunction was indicated greater in school-aged children and females. Nutritional dysregulation caused due to deficiency of essential micronutrients (iodine, selenium, iron) as well as a diet high in goitrogen could be a considerable contributing factor in initiation of thyroid dysfunction. Often clinical demonstrations are non-specific (e.g., short stature, constipation), indicating the need of proper diagnostic test instead of relying on usual signs like goiter.

Our study was a hospital based study carried on small sample thus participant were not representative of the whole population. While our study successfully recognized important associations, but due to its observational nature it was not possible to create a direct cause-and-effect link between diet and thyroid dysfunction. Future research should aim to conduct more in-depth analyses by considering diagnostic tests to measure thyroid antibody levels and concentrations of iodine, selenium, and iron in blood serum which would provide direct association of nutrient status, autoimmunity, and thyroid function. Moreover, Public initiatives should be taken that promote awareness related management of balanced nutrition to prevent early thyroid dysfunction in children. Furthermore, female children who possess non-specific symptoms like poor growth, constipation, fatigue should go for routine thyroid function screenings.

REFERENCES

- 1. Mohammud Habash, M. (2021). Prevalence of Thyroid Defects in Diyala, Iraq. Medico-Legal Update, 21(3).
- 2. Dunn, D., & Turner, C. (2016). Hypothyroidism in women. Nursing for women's health, 20(1), 93-98.
- 3. Fatourechi, V., Klee, G. G., Grebe, S. K., Bahn, R. S., Brennan, M. D., Hay, I. D., ... Morris III, J. C. (2019). Effects of reducing the upper limit of normal TSH values. Jama, 290(24), 3195-3196.
- 4. Skarpa, V., Kousta, E., Tertipi, A., Anyfandakis, K., Vakaki, M., Dolianiti, M., . . . Papathanasiou, A. (2011). Epidemiological characteristics of children with autoimmune thyroid disease. Hormones, 10, 207-214.
- Ambekar, S. A., Wattamwar, P. P., Siddiqui, A. A., & Zuberi, H. R. (2015). Hemiagenesis of Thyroid Gland: A Case Report. International Journal of Scientific and Research Publications.
- 6. Cate, F. L., Moffett, C., Grotowski, A. M., Grenache, D. G., Hartmann, K. E., & Woodworth, A. (2013). Analytical and clinical validation of the Immulite
- Nafisa, A., Ikram, N., Khursheed, S., Anjum, R., & Akhtar, N. (2021). Epidemiologic Profile of Thyroid Disorders in a Tertiary Care Hospital, a Five Years Analysis. Journal of Rawalpindi Medical College, 25(4), 466-471.
- 8. Pirahanchi, Y., Toro, F., & Jialal, I. (2018). Physiology, thyroid stimulating hormone.
- 9. Knight, J., Andrade, M., & Bayram-Weston, Z. (2021). Endocrine system 3: thyroid and parathyroid glands. Nursing Times, 117(7), 46-50.
- 10. Beck-Peccoz, P., Rodari, G., Giavoli, C., & Lania, A. (2017). Central hypothyroidism—a neglected thyroid disorder. Nature reviews endocrinology, 13(10), 588-598.
- 11. Schneider, C., Feller, M., Bauer, D. C., Collet, T.-H., da

Journal of Xi'an Shiyou University, Natural Science Edition

- Costa, B. R., Auer, R., . . . O'Leary, P. C. (2018). Initial evaluation of thyroid dysfunction-Are simultaneous TSH and fT4 tests necessary? PLoS One, 13(4), e0196631.
- Desai, M. P., Upadhye, P., Colaco, M. P., Mehre, M., Naik, S. P., Vaz, F. E., ... & Thomas, M. (1994). Neonatal screening for congenital hypothyroidism using the filter paper thyroxine technique. The Indian journal of medical research, 100, 36-42.
- 13. Desai, M. P. (1997). Disorders of thyroid gland in India. The Indian Journal of Pediatrics, 64(1), 11-20.
- 14. World Health Organization, & International Council for Control of Iodine Deficiency Disorders. (1994). Indicators for assessing iodine deficiency disorders and their control through salt iodization. In Indicators for assessing iodine deficiency disorders and their control through salt iodization
- Jiskra, J., Paleček, J., Attanasio, R., Hegedüs, L., Nagy, E. V., Papini, E., ... & Kršek, M. (2022). Use of thyroid hormones in hypothyroid and euthyroid patients: a 2020 THESIS questionnaire survey of members of the Czech Society of Endocrinology. BMC Endocrine Disorders, 22(1), 117.
- 16. Léger, J., Olivieri, A., Donaldson, M., Torresani, T., Krude, H., Van Vliet, G., & ESPE-PES-SLEP-JSPE-APEG-APPES-ISPAE, and the Congenital Hypothyroidism Consensus Conference Group. (2014). European Society for Paediatric Endocrinology consensus guidelines on screening, diagnosis, and management of congenital hypothyroidism. The Journal of Clinical Endocrinology & Metabolism, 99(2), 363-384.
- 17. Jacobson, D. L., Gange, S. J., Rose, N. R., & Graham, N. M. (1997). Epidemiology and estimated population burden of selected autoimmune diseases in the United States. Clinical immunology and immunopathology, 84(3), 223-243.
- 18. Pearce, E. N., Farwell, A. P., & Braverman, L. E. (2003). Thyroiditis. New England Journal of Medicine, 348(26), 2646-2655.
- 19. Felker, P., Bunch, R., & Leung, A. M. (2016). Concentrations of thiocyanate and goitrin in human plasma, their precursor concentrations in brassica vegetables, and associated potential risk for hypothyroidism. Nutrition reviews, 74(4), 248-258.
- 20. Zimmermann, M. B., & Boelaert, K. (2015). Iodine deficiency and thyroid disorders. The lancet Diabetes & endocrinology, 3(4), 286-295.
- 21. Schomburg, L. (2012). Selenium, selenoproteins and the thyroid gland: interactions in health and disease. Nature reviews endocrinology, 8(3), 160-171.
- 22. Hess, S. Y. (2010). The impact of common micronutrient deficiencies on iodine and thyroid metabolism: the evidence from human studies. Best Practice & Research Clinical Endocrinology & Metabolism, 24(1), 117-132.
- 23. Salerno, M., Capalbo, D., Cerbone, M., & De Luca, F. (2016). Subclinical hypothyroidism in childhood—current knowledge and open issues. Nature Reviews Endocrinology, 12(12), 734-746.
- 24. Sosnowska-Sienkiewicz, P., Danielewicz, D., Januszkiewicz-Lewandowska, D., Rusak, P., Anderko, I., Rzepecki, M., ... & Mańkowski, P. (2024). Thyroid diseases in children and adolescents requiring surgical treatment—indications, techniques, results, and complications based on 10 years of the single center's own experience. Frontiers in Endocrinology, 14, 1301191.

AUTHORS

ISSN: 1673-064X

First Author – Sadaf Sultana, M. Phil Scholar, University Institute of Biochemistry and Biotechnology, PMAS Arid Agriculture University Rawalpindi,

Second Author – Muhammad Bilal, Ph. D., University Institute of Biochemistry and Biotechnology, PMAS Arid Agriculture University Rawalpindi & School of Life Sciences, University of Science and Technology of China,

Third Author – Feroza Hamid Wattoo, Associate Professor, University Institute of Biochemistry and Biotechnology, PMAS Arid Agriculture University Rawalpindi,

Forth Author – Asma Nafisa, Assistant Professor, Rawalpindi Medical University,

Fifth Author – Syeda Saman Batool, M. Phil Scholar, University Institute of Biochemistry and Biotechnology, PMAS Arid Agriculture University Rawalpindi,

Sixth Author – Maryam Sadaqat, M. Phil Scholar, University Institute of Biochemistry and Biotechnology, PMAS Arid Agriculture University Rawalpindi.

Seventh Author – Ahmad Faraz, M. Phil Scholar, University Institute of Biochemistry and Biotechnology, PMAS Arid Agriculture University Rawalpindi,

Correspondence Author - Muhammad Bilal,