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THE STUDY OF ROLE APELIN, GLUCOSE, VITAMIN D AND SOME BIOCHEMICAL PARAMETERS IN CHILDREN WITH KFD

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THE STUDY OF ROLE APELIN, GLUCOSE, VITAMIN D AND SOME BIOCHEMICAL PARAMETERS IN CHILDREN WITH KFD

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January 2023

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ABSTRACT

THE STUDY OF ROLE APELIN, GLUCOSE, VITAMIN D AND SOME BIOCHEMICAL PARAMETERS IN CHILDREN WITH KFD

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Master of Science in Chemistry

Advisor: Prof. Dr. Volkan EYÜPOĞLU

January 2023

At the current research we attempt to study the change in apelin, vitamin D, and arginase as principal biomarkers in children and teenagers with renal failure (RF) disease. Two groups of control were enrolled according to their age to get an optimum comparison strategy, leaving the effect of age (especially the changes that happen during puberty) out of the study. The results have shown non-significant change of apelin level between patients and control neither in children nor in teenagers. While serum vitamin D level was reduced in RF patients of both children and teenagers compared to the corresponded control, whereas the level of vitamin D was non-significantly differed between children and teenagers. Serum arginase level was increased significantly in RF patients of both children and teenagers compared to control, and the teenagers have shown significant higher levels of arginase compared to children with RF disease. Vitamin D was correlated negatively with arginase in children with RF disease. At last, vitamin D and arginase can be used to monitor the RF cases in children and young people to avoid the health consequences arises from the upregulation of these two parameters, while apelin was found to be unassociated with the pathophysiology of renal failure in children and teenagers.

2023, 52 pages

Keywords: Apelin, Vitamin D, Children, CKD, Lipid profile

ÖZET

KFD'Lİ ÇOCUKLARDA APELIN, GLUKOZ, D VİTAMİNİ VE BAZI BİYOKİMYASAL PARAMETRELERİN ROLÜNÜN İNCELENMESİ

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Mevcut araştırmada, böbrek yetmezliği (RF) hastalığı olan çocuk ve gençlerde başlıca biyobelirteçler olarak apelin, D vitamini ve arginazdaki değişimi incelemeye çalışıyoruz. Yaşın etkisini (özellikle ergenlik döneminde meydana gelen değişiklikleri) çalışmanın dışında bırakarak, optimum bir karşılaştırma stratejisi elde etmek için yaşlarına göre iki kontrol grubu kaydedildi. Sonuçlar, ne çocuklarda ne de gençlerde hastalar ve kontroller arasında anlamlı olmayan apelin seviyesi değişikliği göstermiştir. Hem çocuk hem de ergen RF hastalarında serum D vitamini düzeyi karşılık gelen kontrole göre azalırken, D vitamini düzeyi çocuklar ve ergenler arasında anlamlı olmayan bir şekilde farklıydı. Hem çocuklarda hem de gençlerde RF hastalarında serum arginaz düzeyi, kontrole kıyasla önemli ölçüde arttı ve gençler, RF hastalığı olan çocuklara kıyasla önemli ölçüde daha yüksek arginaz seviyeleri gösterdi. D vitamini, RF hastalığı olan çocuklarda arginaz ile negatif korelasyon gösterdi. Son olarak, D vitamini ve arginaz, bu iki parametrenin yükselmesinden kaynaklanan sağlık sonuçlarından kaçınmak için çocuklarda ve gençlerde RF vakalarını izlemek için kullanılabilirken, apelinin çocuklarda böbrek yetmezliğinin patofizyolojisi ile ilişkili olmadığı bulunmuştur. gençler.

2023, 52 sayfa

Anahtar Kelimeler: Apelin, D Vitamini, Çocuklar, KBH, Lipit profili

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LIST OF SYMBOLS

-	Minus
%	Percent
/	Divide
+	Plus
<	Lesser than
>	Greater than
µL	Microliter
dL	Deciliter
IU	International Unit
L	Litre
m^2	Meter square
mg	Milligram
min	Minute
mL	Milliliter
mmol	Milimole
nm	Nanometer
ºC	Degrees Celsius
pg	Picogram
rpm	Revolution per minute

LIST OF ABBREVIATIONS

APLN	Apelin
ARG1	Arginase 1
ARG2	Arginase 2
ASL	Argininosuccinate lyase
ASS	Argininosuccinate synthase
CKD	Chronic kidney disease
CKiD	Chronic kidney disease in children
CPS-1	Carbamoyl phosphate synthase-1
DKD	Diabetic kidney disease
ESRD	End stage kidney disease
GFR	Glomerular filtration rate
GH	Growth hormone
HD	Hemodialysis
HDL	High-density lipoprotein
IGF1	Insulin-like growth factor 1
IOM	Institute of medicine
MBD	Mineral and bone disorder
OCT	Ornithine carbamoyl-transferase
PD	Peritoneal dialysis
PGC1α	Proliferator-activated receptor gamma coactivator-1α
RAS	Rennin-angiotensin system
RCTs	Randomised controlled trials
RRT	Renal replacement therapy
T2D	Type 2 diabetes
TC	Total cholesterol

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1. INTRODUCTION

The 2002 KDOQI Guidelines provided the initial definition of chronic kidney disease (CKD), which was later supported with slight adjustments at the 2012 KDIGO Controversies Conference (Kasiske *et al.* 2013). The pediatric nephrology community has seen limits as well as advantages from these categories, which were relevant to children. The CKD grading system is based on arbitrary eGFR thresholds, only applies to children older than 2 years old, ignores age- and sex-related changes in GFR, gives more weight to albuminuria while the majority of children have non-glomerular diseases, and stages 1-2 would be better defined by associated abnormalities rather than being labeled as childhood CKD. These are some of the criticisms that can be made of the system. But the CKD categories, which were widely embraced, gave order to the disorder of CKD nosology, enhanced pediatricians' comprehension of the issue, stoked interest in systematic kidney disease diagnosis, and prompted clinical and epidemiological research on pediatric CKD (Querfeld *et al.* 2010).

The adipose tissue-derived peptide known as apelin (APLN) regulates neuroendocrine function, eating behavior, and energy balance in the CNS and peripheral organs (O'Carroll *et al.* 2000). There is conflicting information about the significance of APLN, particularly in children, and its relationship to obesity and cardiometabolic risk factors. According to certain research, levels are higher in obese persons than in subjects of normal weight. Other research, however, showed no appreciable variations or even lower levels in obese youngsters compared to healthy ones (Tapan *et al.* 2010).

For the metabolism of vitamin D, normal renal function is essential. Patients with chronic kidney disease (CKD) have a significant prevalence of vitamin D insufficiency (20%-85%) (Satirapoj *et al.* 2013). The majority of patients died from cardiovascular causes, according to a prospective cohort analysis of 444 patients with an eGFR of less than 60 mL/min/1.73 m2 (9.4 years of follow-up) (Pilz *et al.* 2011).

A different research found a correlation between urinary albumin loss and urine DBP losses in children with CKD stages 4-5 who were receiving peritoneal dialysis (PD). They concluded that both urine and dialysate albumin losses are mimicked by peritoneal DBP losses, do not cause vitamin D insufficiency in children receiving PD, they are linked to prolonged dialysis vintages (Prytuła *et al.* 2012).

Arginase, a widely distributed metalloenzyme with the L-arginine hydrolase activity that has been discovered in vertebrates, invertebrates, yeasts, bacteria, and plants, plays a crucial role in both pathological and physiological circumstances (Dzik 2012). (Caldwell *et al.* 2018). Arginase comes in two different isoforms: arginase 1 (ARG1), which is expressed in the cytosol, and arginase 2, which is mostly found in the mitochondria. Despite having about 60% homology, the two isoforms exhibit 100% homology in the regions crucial for catalytic activity (Vockley *et al.* 1996), and show comparable enzymatic activity. However, the tissue distribution and physiological roles of the two isoforms vary (Pernow and Jung 2013).

Dyslipidemia is one of the co-morbidities seen in children with CKD/ESRD. The incidence of dyslipidemias in children with ESRD and CKD is substantial (39-65%), but it greatly depends on the type and stage of the illness, as well as the origin and vintage of the CKD (Khandelwal *et al.* 2016). A comparison of renal function and blood lipid levels was made as part of (CKiD) research. Hypertriglyceridemia was the most prevalent kind of dyslipidemia. They discovered an inverse association between serum total cholesterol (TC) and TG levels and renal function; that is, when GFR decreases, levels of TC and TG often rise. On the other hand, there is a direct connection between HDL-C and GFR (Wong *et al.* 2012).

1.1 Aim of Study

Assessment of the levels of apelin protein with vitamin D and research on the possible role of them with some chemical tests in the early treatment and the diagnosis of renal failure disease in children and compare them with healthy controls and use these apelin protein with vitamin D as an indicator of the renal failure disease in the children.

2. LITERATURE REVIEW

2.1 Chronic Kidney Disease

Regardless of the underlying etiology, CKD is a clinical illness that can lead to end-stage renal disease since it is characterized by a slow, irreversible decrease of kidney function (ESRD) (Levin *et al.* 2013). CKD affects 8–18% of adults globally, making it a serious public health issue (Webster *et al.* 2017, Stern-Zimmer *et al.* 2021) as well as a pediatric frequency of 15–74.7 cases per million kids (Warady and Chadha 2007). According to registry statistics from European nations, 56–96 children per million have CKD (Harambat *et al.* 2012). About its genuine impact on children, especially in environments with little resources, very little is understood. A deadly condition with significant morbidity and fatality rates is ESRD. Clinical characteristics of childhood CKD, such as growth failure and psychosocial problems, have a major negative influence on quality of life. CKD-related cardiovascular problems cause morbidity in young adults (Becherucci *et al.* 2016). The true impact of CKD on children in developing nations is unknown because, with the exception of a few tertiary centers, the majority of young patients go undiagnosed in the early stages, and many more pass away before receiving (RRT), which is expensive and challenging to administer to children without necessary infrastructure for health workers and systems.

The healthcare system and those who treat children with CKD have particular challenges since they must address both the basic kidney condition and the numerous extra-renal symptoms that complicate therapy (Harambat *et al.* 212). The majority of children with CKD and ESRD in Pakistan, as in other poor nations, appear in advanced stages. Even in wealthy nations, the death rate for children in this age group is 30-150 times greater than for children in good condition (Collins *et al.* 2015).

2.2 Children with Chronic Kidney Disease

Over the past 20 years, tremendous progress has been made in our knowledge of the cause and treatment of growth failure in children with (CKD). However, in comparison to children with healthy age- and sex-matched controls, 40% of children with (ESRD) have a lower ultimate height (Harambat *et al.* 2014). Short height is linked to higher mortality and lowers quality of life, self-esteem, and social reintegration (Mekahli *et al.* 2014).

Multifactorial factors such as malnutrition, bone disorders (MBD), intrauterine growth restriction, mineral, metabolic acidosis, electrolyte loss, and disruptions of the somatotropic and gonadotropic hormone axis are the causes of growth failure in CKD (Figure 2.1). Advanced chronic kidney disease (CKD) is characterized by a lack of the functional insulin-like growth factor 1 and a condition of growth hormone (GH) insensitivity (IGF1) (Tönshoff *et al.* 2007).

Recombinant human GH, often known as GH, can be used to treat this GH insensitivity. GH enhances longitudinal development, normalizes somatomedin bioactivity, stimulates IGF1 production, and presumably increases adult height. As a result, GH has received a license in North America, Europe, and several other high-income nations for the treatment of CKD-related growth failure (Rees 2016).

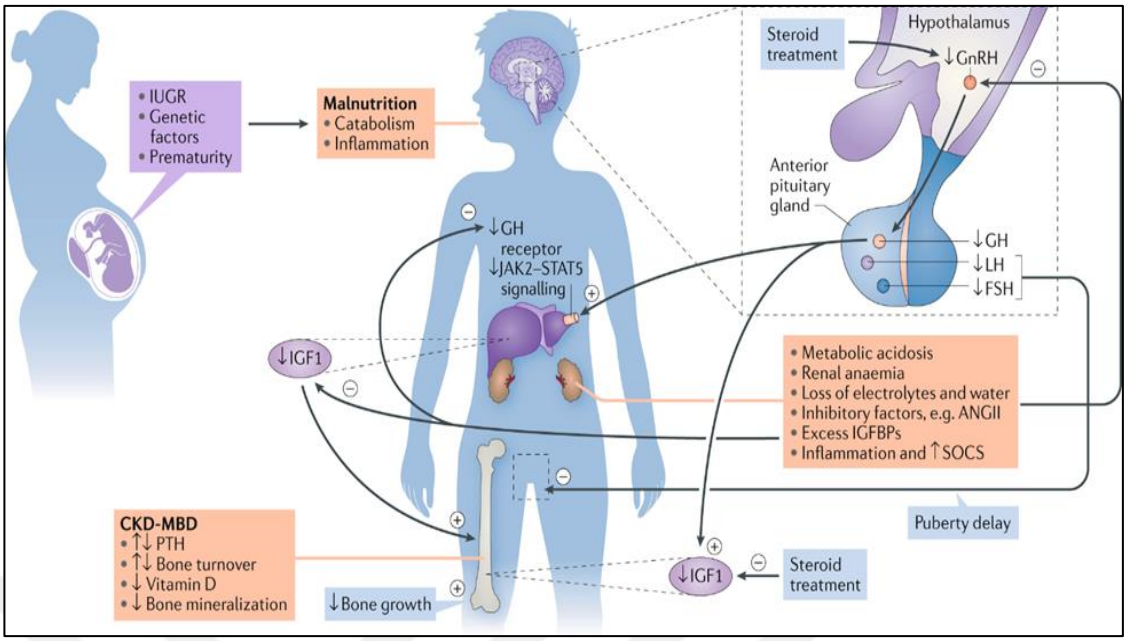


Figure 2.1 Growth failure in children with CKD is caused by a number of factors (Drube *et al.* 2019)

2.2.1 Epidemiology of chronic renal failure in children

The KDIGO recommendations state that the presence of structural or functional renal impairment or a prolonged fall in (GFR) to less than 60 mL/min/1.73 m2 of body surface area are both indicators of CKD (Levin *et al.* 2013). As a result, rather than designating a distinct alteration in renal function, whether in infants or adults, the term "CKD" characterizes renal failure as a continuum (Cattran *et al.* 2012). This makes it exceedingly challenging to research the CKD epidemiology. Furthermore, because CKD is frequently clinically silent, particularly in early stages, epidemiologic statistics on its incidence and prevalence may be inaccurate. This is partly due to the previous lack of a standardized definition of the CKD and a well defined scale for grading its severity, which has only recently, at least in part been addressed with the establishment of KDIGO recommendations. For all of these reasons, estimations of CKD are often not population-based research and instead include individuals with moderate to severe CKD or (ESRD). Furthermore, the use of small reference groups typically places restrictions on childhood CKD registries. Despite these drawbacks, it is estimated that during stages 3-5 of the disease, the paediatric incidence of CKD in Europe is 11–12 per million of

the age-related population (pmarp), whereas the prevalence is 55–60 pmarp (Harambat *et al.* 2011). Population-based registries of other western nations show comparable numbers, although few of them have access to exact information on the prevalence and incidence of pre-terminal CKD. If various age groups are studied, certain variances could show up. Paediatric CKD incidence increased gradually during the 1980s, then the somewhat through the first decade of twenty-first century (Collins *et al.* 2006).

In addition, because CKD survival and treatment have considerably improved, the prevalence of the condition has significantly grown (Baum 2010). Patients with ESRD needing renal replacement treatment have received the majority of attention in specific publications on the CKD epidemiology in children (RRT). The prevalence is stated to be 65 pmarp, whereas the median incidence of RRT in children under 20 years old is reported to be 9 pmarp globally (Harambat *et al.* 2014). Additionally, greater incidence and prevalence numbers have been recorded for the USA, perhaps as a result of RRT being initiated sooner and at higher GFR levels than in other wealthy nations.

2.2.2 Peritoneal dialysis in children

PD is a life saving of kidney replacement procedure that takes advantage of the peritoneum's characteristics as a semipermeable biomembrane to help patients with kidney failure eliminate excess solutes, toxins, and water. In children with (CKD), PD is the preferred and most often utilized treatment option, particularly for young children and newborns in whom establishing a long-term vascular access for the hemodialysis can be extremely difficult. High compatibility with education and social life is offered by automated cycler dialysis over night. Additionally, Due to the rarity of conditions needing chronic kidney replacement therapy, there are just a few specialist pediatric dialysis facilities accessible in most nations; distances are frequently great, and many children find it difficult to travel for three follow-up visits per week as required for hemodialysis. In addition, PD is favored in cardiovascular instability patients, which may be made worse by hemodialysis's high rates of the fluid removal and extracorporeal blood volume over a short period of time (Warady *et al.* 2020). When compared to hemodialysis, early survival is better with PD in adults (Van De Luijtgaarden 2016).

2.2.3 Hemodialysis in children

Young children with CKD, who are most likely to receive enteral tube feeding, are less likely to undergo hemodialysis (HD). The tiny stature of young patients presents a unique difficulty for extracorporeal circuit capacity needed as a proportion of blood volume. Having strict fluid management is important, which reduces the amount of food that can be fed. Three times a week hemodialysis creates a requirement for stricter electrolyte management, and using a lower electrolyte formula may be advised. For the youngest patients, some hospitals extend hemodialysis to as much as six times per week. Only a few facilities in North America provide home hemodialysis, but it is another alternative to allow for the liberalization of the enteral feeding since more frequent dialysis often results in clearance, better blood pressure, and serum potassium levels (Kaur and Davenport 2014).

2.2.4 Evaluation and treatment of CKD

A normal eGFR and the presence of microalbuminuria define the early stage of CKD. This mild form of CKD bears similar risks developing of ESRD and CV illness as stage 3 CKD and has been linked to a 25-40 fold increase in ESRD risk (De-Jong *et al.* 2008). The risk of microalbuminuria was not significantly different between Whites and Mexican Americans in a study of people with optimal and high-normal blood pressure, but it was significantly higher (OR 1.30; CI 1.04-1.64) in African Americans and trending toward increased risk (OR 1.16; CI 0.90-1.51) in Mexican Americans (Knight *et al.* 2003).

When compared to Whites, African Americans have an overall incidence of hypertension-related ESRD that is nearly five times higher than that of Whites. Young American-African men between the ages of 44-20 also have > 15 times higher rates of the hypertension related ESRD than do their White counterparts. This may be partially attributed to the higher risk of the proteinuria that Americans-African exhibit at any given elevated levels of BP (Norris and Agodoa 2005).

The course of CKD is accelerated by cardiovascular risk factors and the existence of CV illness, which also increases the risk of death (Chobanian *et al.* 2003). C Contrarily, CKD in all phases is linked to a higher risk of the complications and CV mortality (Baigent *et al.* 2000). The crucial function of the (RAS) in the hypertension controls and the mitigation of problems connected to the hypertension is at the center of a significant percentage of the etiologic hypotheses and treatment approaches in underprivileged populations. Because of the known involvement of RAS in CKD development, populations with low renin hypertension were expected to have a reduced risk of end-organ damage from hypertension (Luft *et al.* 1979).

2.3 Diabetes and Chronic Kidney Disease

Type 1 diabetes (T1D), which has an annual percentage change of 1.9%, and (T2D), which has an annual percentage change of 4.8%, have both increased in prevalence among children and adolescents. This increase is much more prominent among racial and ethnic minorities (Kahkoska *et al.* 2018) and for T2D, coincides with the rise in obesity prevalence. Additionally, children and adolescents with diabetes experience a more aggressive clinical course compared to adults, which is characterized by a muted response to existing therapies (Hannon *et al.* 2020), furthermore, quicker evolution of insulin resistance, loss of β-cell function, and end-organ problems (Bjornstad *et al.* 2018).

2.4 Dyslipidemia in Children

Studies in both adults and children have demonstrated that dyslipidemia, which is frequently present in people with CKD, is caused by problems with lipoprotein metabolism (Saland and Ginsberg 2007). A cross-sectional examination of the lipid measures was carried out in 391 individuals with the CKD to ascertain the frequency and pattern of the dyslipidemia in CKD kids (Saland *et al.* 2010).

Of the CKiD cohort, 32% (n=126) had hypertriglyceridemia. Children with a normal BMI had levels of triglyceride that were on average 30% higher than those with overweight or obesity, and 61% of kids with proteinuria in the nephrotic range has hypertri glyceridemia. The CKiD cohort also had other lipid abnormalities, with 16% having levels of non-HDL-C and 21% having low levels of HDL-C; these abnormalities were linked to obesity, older age, and reduced GFR (Saland *et al.* 2010).

A rising incidence of dyslipidemia was likewise associated with increasing levels of proteinuria. Our results corroborate recommendations to screen children with CKD for dyslipidemia in order to detect this risk factor early, direct medication, and ultimately improve cardiovascular health given the high prevalence of dyslipidemia in this population (Mitsnefes *et al.* 2010).

2.5 Apelin

Apelin (APLN), a 77 amino acid bioactive neuropeptide, was initially discovered in bovine stomach tissue and is an endogenous ligand for the APJ receptors (APLNR). The APLN prepeptide was broken down and processed by protease to produce a number of molecular forms, the most active of which is apelin-13. Using APJ signaling, the new adipocytokine apelin affects G-protein-coupled receptors (APJ) (Yamaleyeva *et al.* 2019).

protein receptor APJ has no introns in the coding region and shares a sequence with the angiotensin receptor type 1 (AT1) gene. It mostly binds to apelin-13 in particular (He et al. 2015). Other than APLN, a number of other organs and tissues, such as retinal endothelium, cardiovascular system, blood vessels, adipose tissue, the brain, heart, and skin, can also exhibit APJ (Mughal and O'Rourke 2018). The apelin/APJ system is well known to have a crucial physiological role in pain, energy metabolism, neovascularization, and cardiovascular function (Lv *et al.* 2020) and is involved in diabetes, the metabolic syndrome, cardiovascular disease (Li *et al.* 2018). There is evidence to support certain claims that APLN has high antioxidant action.

2.5.1 Apelin structure/function

Each apelin isoform, including apelin-36, apelin-17, apelin-13, (pGlu)apelin-13, apelin-55, and apelin-12, has a random coil conformation, devoid of any distinguishing secondary structure and having no distinct three-dimensional shape (Narayanan *et al.* 2015). Nevertheless, the APJ receptor appears to be activated similarly by each isoform (Shin *et al.* 2017).

This is explained by the relatively novel mechanism of apelin peptide-APJ receptor binding. Abelin peptides assume a bent shape when they connect to the receptor. They attach at two distinct locations via polar and hydrophobic interactions. It is the unique observation of a GPCR demonstrating dual-binding to a single endogenous ligand, with the remaining portion of the peptide folding around 90 degrees into a second binding site upon interaction of the N-terminus (Ma et al., 2017). The most physiologically active isoforms of apelin include apelin-13 (Figure 2.2), (pGlu)-apelin-13, and apelin-12, which have considerably better effectiveness at the APJ receptor than the longer isoforms. This is surprising because apelin has identical receptor binding kinetics regardless of its shorter length. However, compared to the shorter isoforms, apelin-36 seems to bind to the APJ receptor for a substantially longer period of time (Hosoya *et al.* 2000).

The longer apelin-36 peptide has more positively charged amino acids that interact with negatively charged APJ receptor regions, which may be the cause of the increase in receptor occupancy (Ma *et al.* 2017). A variety of downstream actions, such as the suppression of cAMP and the activation of ERK and Akt, are produced when the APJ receptor connects predominantly to Gαi/o (Masri *et al.* 2006). Although the specific mechanism has not been established, activation of the APJ receptor similarly raises intracellular Ca2+ (O'Harte *et al.* 2018).

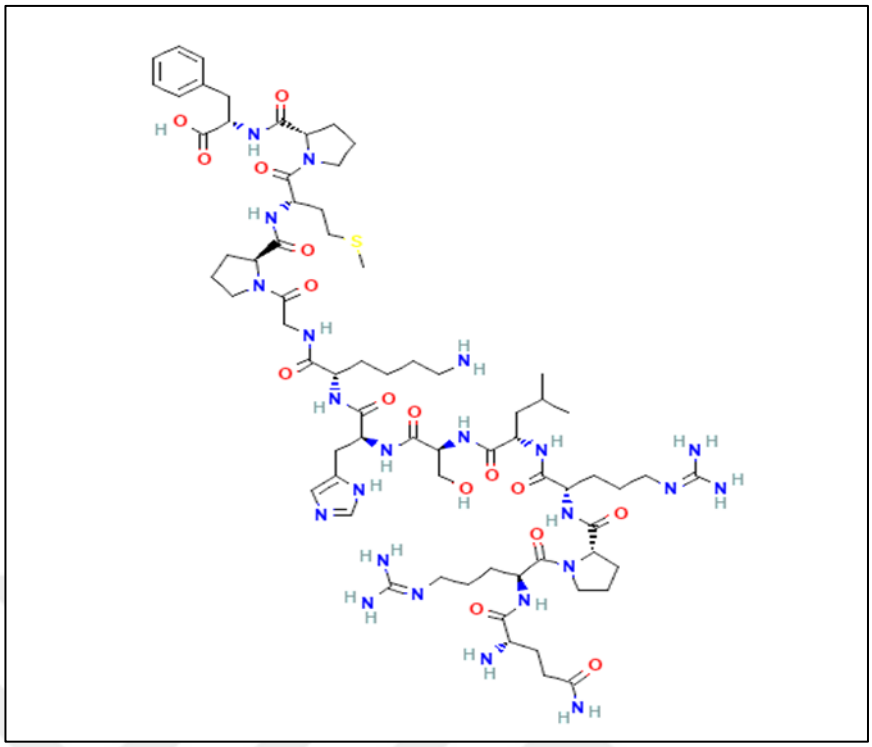


Figure 2.2 Apelin-13 structure

2.5.2 In adipose tissue, apelin expression and secretion are controlled

Insulin was the first gene that was identified as controlling apelin production and secretion in adipocytes. Numerous mice models of hyperinsulinemic obesity show elevated apelin expression in adipocytes as well as plasma concentrations. The production and secretion pattern of apelin in adipocytes is regulated by insulinemia in mice during fasting and after refeeding. Human isolated adipocytes and adipocyte cell lines both exhibit enhanced apelin gene 3 expression and secretion after insulin therapy (Boucher *et al.* 2005). Adipogenesis has also been demonstrated to boost apelin expression (Zhu *et al.* 2015).

Since insulin, growth hormone (GH), inflammatory cytokines TNF and LPS have all been identified as positive regulators of the expression of the apelin gene in adipocytes or adipose tissue (Geurts *et al.* 2011). There are several of common mechanisms that have been identified to activate the apelin gene in adipocyte cell lines, such as an insulin, TNF, and GH-dependent PI3K pathway or insulin- and TNF-activated MAPK,

but there are also differences. It has also been demonstrated that excessive PGC1 expression boosts the expression and release of the apelin gene in human adipocytes, which is a crucial regulator of cellular energy balance in oxidative tissues (Mazzucotelli *et al.* 2008).

Conversely, it is difficult to find agents that inhibit the expression of apelin in adipocytes. Through the activation of p38 MAPK, the use of aldosterone and glucocorticoids in 3T3-L1 cells reduced the amounts of apelin mRNA (Jiang *et al.* 2013).

2.5.3 Apelin in kidney physiology

Studies on the function of apelin in human renal physiology are sparse despite the fact that it is abundantly expressed in the human kidney. According to research on animals, apelin directly affects the kidney, influencing renal hemodynamics and fluid equilibrium. Glomerular hemodynamics: RAS, which controls both GFR and glomerular perfusi, tightly controls glomerular blood flow. The RAS and the apelin system also interact (Figure 2.3). Ang II vasoconstricts both the efferent and afferent arterioles by raising intracellular calcium levels, with the efferent arteriole being the one that is more strongly affected. Apeline causes a significant decrease in intracellular calcium levels while reversing the vasoconstriction that Ang II caused in the afferent and efferent glomerular arterioles of rats. A healthy endothelium and NO synthesis are required for apelin's vasodilatory effect (Hus-Citharel *et al.* 2008). Apeline-induced vasodilatation may encourage diuresis by increasing renal medullary blood flow since the efferent arteriole supplies the vasa recta (Figure 2.3).

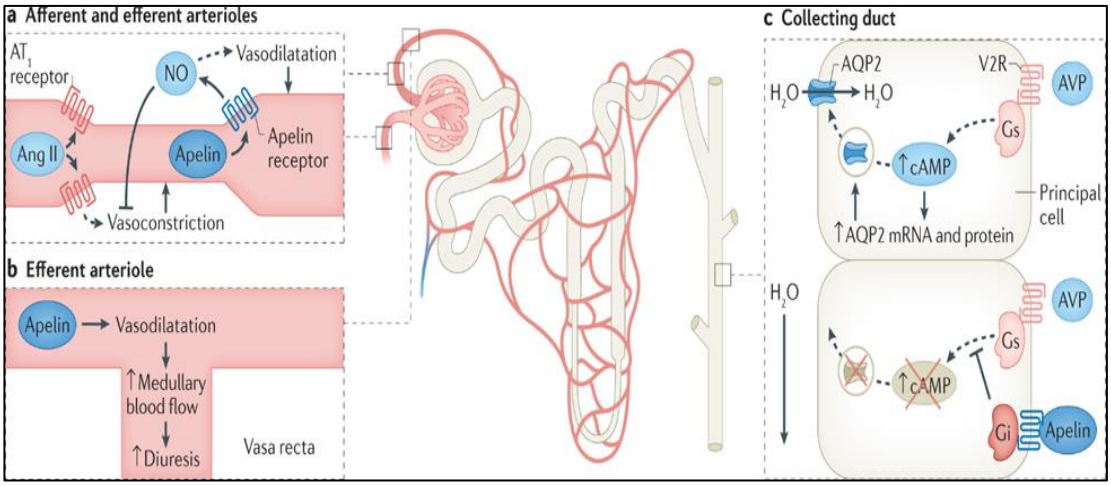


Figure 2.3 The actions of apelin in the nephron (Chapman *et al.* 2021)

2.6 Vitamin D

Vitamin D has the ability to prevent a number of ailments and is a key factor in the body's control of calcium and phosphate (Borges *et al.* 2020). Despite the fact that sunlight is the main source of vitamin D production in humans (Webb and Holick 1988), This might not be enough for certain people, for instance if they are unable to walk outside (like the elderly) or if their clothing is opaque and covers the bulk of their skin. Additionally, it is well-known that those with the darker skin types do not absorb UV radiation's vitamin D precursors as efficiently as those with lighter skin tones do (Cashman *et al.* 2017). In fact, it has been noted that darker skinned individuals need almost three times as much sun exposure as Caucasians do in order to experience the same changes in serum 25-hydroxyvitamin D (25(OH)D) levels. Additionally, it has been noted that African Americans' levels 25(OH)D are positively correlated with a number of health outcomes, such as Alzheimer's disease and multiple sclerosis (Ames *et al.* 2021).

Numerous studies have previously looked at the effects of vitamin D supplementation in persons with Asian heritage and Black (Grønborg *et al.* 2020). Several of these studies have also evaluated changes in serum levels of 25(OH)D in individuals with various

skin tones after vitamin D administration, revealing substantial benefits (Tripkovic *et al.* 2017).

2.6.1 Chronic kidney disease with vitamin D deficiency

The ideal 25(OH)D level is a matter of debate. The Institute of Medicine (IOM) published a study in 2011 on Dietary Requirements for Calcium and Vitamin D that classified vitamin D inadequacy as levels of 25(OH)D < 20 ng/mL, which is generally regarded as acceptable for bone health. The Endocrine Society believes that a level of 30 ng/mL is the lowest tolerable level and does not concur with the IOM's recommendations. This is supported by research that found a 33% decrease in osteoporotic fractures, when levels of the 25(OH)D were increased 20 to 21–29 ng/mL (Heaney and Holick 2019), as well as meta-analyses that found no appreciable decrease in the fracture rates. Having levels of the 25(OH)D between 30 and 20 ng/mL (Bischoff-Ferrari et al. 2009), and aberrant bone suture appearance in 6 of 28 participants in an autopsy investigation (Bremel *et al.* 2010). Values between 21 - 29ng/mL are regarded inadequate, while 20 ng/mL levels below are considered deficient, even though the concept of vitamin D inadequacy is disputed (Holick *et al.* 2011).

2.7 Arginase

Arginase is the sixth and final enzyme of the urea cycle and converts arginine to urea and ornithine. Urea is excreted from the body, and ornithine is recycled. A block in this enzyme activity results in high levels of arginine. However, arginase deficiency is the least likely of the urea cycle disorders to cause neonatal hyperammonemia. The clinical presentation and progression were described previously. In most patients, arginine level alone is elevated in plasma, although glutamine level may also rise when ammonia levels are elevated. Ammonia levels are often normal between acute episodes (Martens *et al.* 2014).

In contrast to other urea cycle disorders, the proximate toxin may be arginine and not ammonia or glutamine, and this may account for the different symptoms. The liver form of arginase (ARGI) is absent in red blood cells. A second arginase (ARGII) is present at lower levels in the mitochondria of many tissues and may in part be responsible for the milder clinical expression of arginase deficiency. The ARGI gene has been cloned, and a number of mutations are known. By analyzing mutations and determining the amounts of arginase in red blood cells collected by percutaneous umbilical blood sample, prenatal diagnosis is achievable. Diagnosis in the newborn should be possible by tandem mass spectrometric analysis of arginine levels in blood (Wetzel *et al.* 2020).

2.7.1 Actions of arginase

The ureohydrolase family of enzymes includes arginase. The last stage of the urea cycle, in which the body eliminates harmful ammonia created by protein catabolism, is catalyzed by arginase in the liver. In particular, the enzyme arginase turns L-arginine into urea and L-ornithine (Figure 2.4).

By using ornithine carbamoyl-transferase and the enzymes (CPS-1), arginine converts ammonia and ornithine into L-citrulline (OCT). To complete the urea cycle, L-citrulline can be converted back into the L-arginine by the enzymes (ASL) and (ASS) (Osowska *et al.* 2004). The majority of the non-hepatic tissues, however, do not contain the whole urea cycle because they lack CPS-1or OCT.

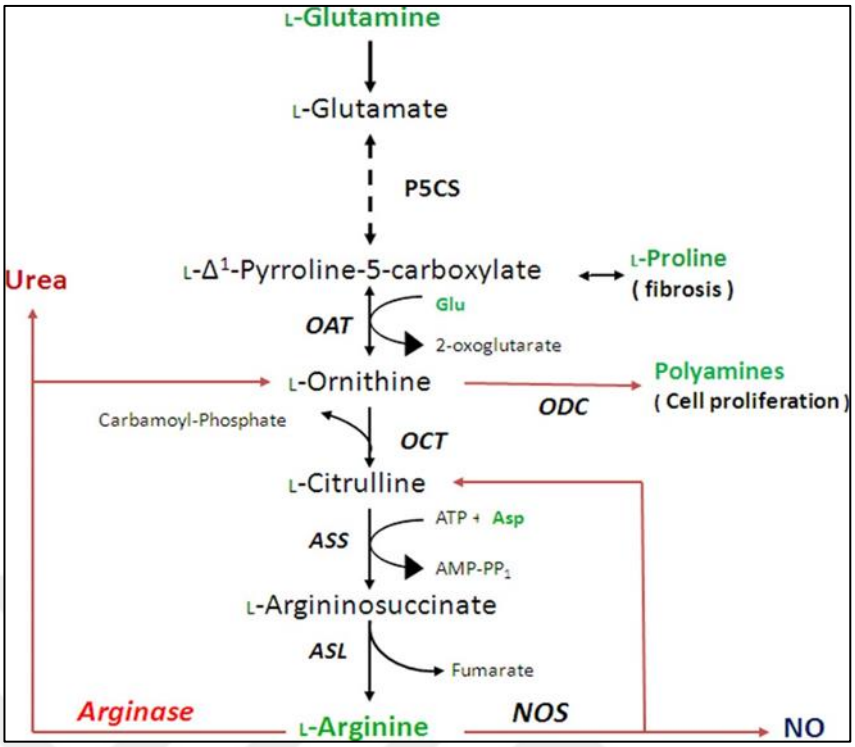


Figure 2.4 L-arginine can be made using the following formula: L-glutamine (Narayanan *et al.* 2013)

Arginase and NOS can compete for the same substrate, L-arginine, if arginase activity is increased. A semi-essential amino acid called L-arginine can cause NOS to become uncoupled if there is inadequate supply to support NOS action (Romero *et al.* 2008).

3. MATERIALS AND METHODS

3.1 Design of Study

This study included children with chronic renal failure, where we divided the study groups as described below, and this study consisted of 160 samples (120 children with RFD and 40 children without RFD as a control). The samples were taken with varying degrees of disease activity. Taking into account the ages (1-16 years), they were divided into children and adolescents. Gender and body mass index were taken into consideration. Apelin protein and glucose along with vitamin D and some biochemical markers were measured in all patients and control groups.

We divided the study into four groups:

- Group A: 40 Control children without RF.
- Group B: 40 Children with RF
- Group C: 40 Control teenagers without RF
- Group D: 40 Teenagers with RF

3.2 The Date and Location of Sample Collection

In this study, samples were collected from the Child Hospital in Baghdad/Iraq from the 8th of April to the 15th of October.

3.3 Excluded Samples

Obesity, cancer and patients who underwent treatment were excluded. Samples over 17 years of age were also excluded

3.4 Sample Collection

In this study, 7 mL of blood was drawn from patients with chronic renal failure. 5 mL was placed in the gel tube and 2 mL in the EDTA tube. The gel tubes were placed in the chamber for 10 minutes, after which the tubes were placed in a centrifuge in order to separate the blood from the serum, after which the serum was placed in Eppendorf tubes, closed and placed in the refrigerator at a temperature of -18 °C.

3.5 Methods

3.5.1 Human total apelin (Apelin) ELISA kit

Apelin ELISA kit uses the quantitative sandwich enzyme immunoassay technology as the basis for the test. Monoclonal antibodies that are focused on Apelin have been pre-coated on the microtiter plate. After adding standards or samples to the microtiter plate wells, apelin will bind to the wells that have already been coated with an antibody if it is present. Apelin-specific horseradish peroxidase (HRP)-conjugated polyclonal antibodies are added to each well in the order to "sandwich" the Apelin immobilized on plate in the order to the quantitatively measure the quantity of Apelin present in samples. After incubation, all unbound components are removed from the microtiter plate's wells by a thorough washing procedure. The addition of substrate solutions to each well follows. Over a brief incubation time, the substrate and enzyme (HRP) are allowed to react. The only wells that will change color are those that have both Apelin and an enzyme-conjugated antibody. The enzyme-substrate reaction is stopped by adding a solution of sulfuric acid, and the color shift at 450 nm is then measured spectrophotometrically. A standard curve that connects the color's intensity (O.D.) to the standard solution's volume is displayed. Interpolating from this standard curve yields the Apelin concentration in each sample.

3.5.2 Human arginase ELISA kit (Arg)

Principle of The Assay: A monoclonal antibody that is specific for Arg has been pre-coated on the microtiter plate included in this kit. The wells of the microtiter plate are then filled with standards or samples, and any Arg present will bind to antibody - coated wells. The HRP-conjugated polyclonal antibodies specific for the (Arg) added to each well in the order to "sandwich" the Arg immobilized on plate and quantify the amount of the (Arg) present in the sample. After the microtiter plate has been incubated, carefully cleaning the wells removes any unattached components.

The substrate solutions A and B are then added to each well. In a brief incubation time, the enzyme (HRP) and substrate are allowed to react. Only the wells with enzyme-conjugated antibody and Arg will show a color shift. A sulphuric acid solution is used to stop the enzyme-substrate reaction, and the color change in spectrophotometrically is detected at a wavelength of 450 nm.

Calculation of results:

1. The quantity in an ambiguous sample can be estimated using this standard curve. Plotting the average O.D. The standard curve is produced by plotting the concentration reached for each of the six standard concentrations (450 nm) against the concentration indicated on the horizontal (X) axis.

2. The mean O.D. values for each sample and standard should be calculated first. Before interpreting results, all values of O.D. are deducted from the values of mean of the zero standard. Create the standard curve using statistical software or graph paper.

3. The quantity in each sample may be calculated by first finding the O.D. value on the Y-axis and then drawing a horizontal line from there to the standard curve. Read the matching concentration by drawing a vertical line to the X-axis at the junction.

4. The outcome can vary depending on the operator, the pipetting and cleaning procedure, the incubation period or temperature, and the kit age. A unique standard curve should be produced for each user.

5. This assay is 0.01 ng/mL of the sensitivity

6. Standard curve (Figure 3.1)

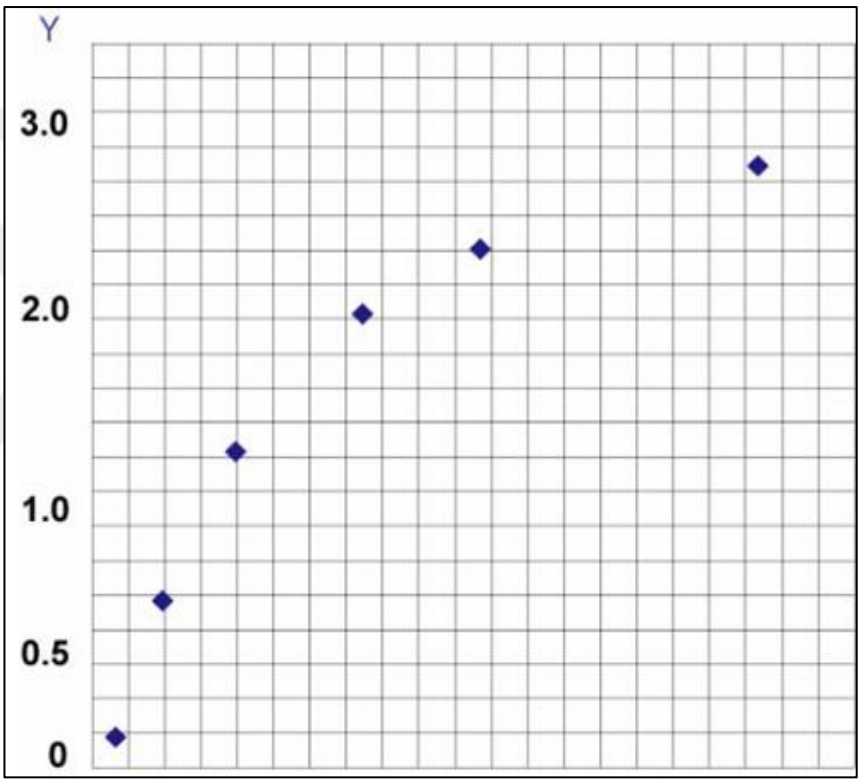


Figure 3.1 Standard curve of arginase

3.6 Materials

3.6.1 Devices used

In this study, as shown in Table 3.1, all devices used in this study were integrated.

Table 3.1 Devices used in this study

No.	Devices	COMPANY	Origin
1	ELISA reader	ELX800, BioTech	USA
2	Visible Spectrophotometer	PD-303 APEL	Japan
3	ELISA washer	ELX50, BioTech	USA
4	Water bath	Memmert	Germany
5	Deep Freeze	GFL	Germany
6	COBAS C111\ full automated	Roche	Switzerland
7	Spinreact/chemistry- full automated	Spinreact	Spain
8	Oven	Memmert	Germany
9	Autoclave	Slamed	Germany

3.6.2 Tools used

In this study, as shown in Table 3.2, all tools used in this study were integrated.

Table 3.2 Tools used in this study

No.	Tools	Company	Origin
1	pH - meter	-	Stuart
2	Different glasses	-	China
3	Gel tube	AFCOVAC	Jordan
4	Medical laboratory centrifugation 5000 rpm	HIGHTOP	China
5	Micropipettes (20-200µL), (100-1000µL), and (1-10 mL)	Slamed	Germany
6	EDTA Tube	AFCOVAC	Jordan
7	Tips (blue, yellow)	AFCOVAC	Jordan

3.6.3 Kits used

In this study, as shown in Table 3.2, all kits used in this study were integrated.

Table 3.3 List of kits used in this study

No.	Kits	REF
1	Human Arginase Elisa Kit (Arg)	MBS720297
2	Determination Cholesterol	03039773 190
3	Human Total Apelin (Apelin) Elisa kit	MBS756419
4	Determination of Triglycerides	MX41031
5	Creatinine	1123005
6	Determination LDL-Cholesterol	07005717 190
7	HbA1c	HBA0202
8	Urea	TK41041
9	Determination HDL-Cholesterol	07528566 190
10	Fasting Glucose	MD41011
11	VLDL	TG/5

4. RESULTS

4.1 Age

The age of children with RF was 5.83±2.41 year, which was non-significant (p=0.468) when statistically compared to the children control without RF (6.48±2.16 year). Additionally, the comparison of age in teenagers with RF (14.48±1.68 year) and control teenagers without RF (14.63±1.64 year) was also non-significant (p=0.987). The graphic illustration of data is seen in Figure 4.1.

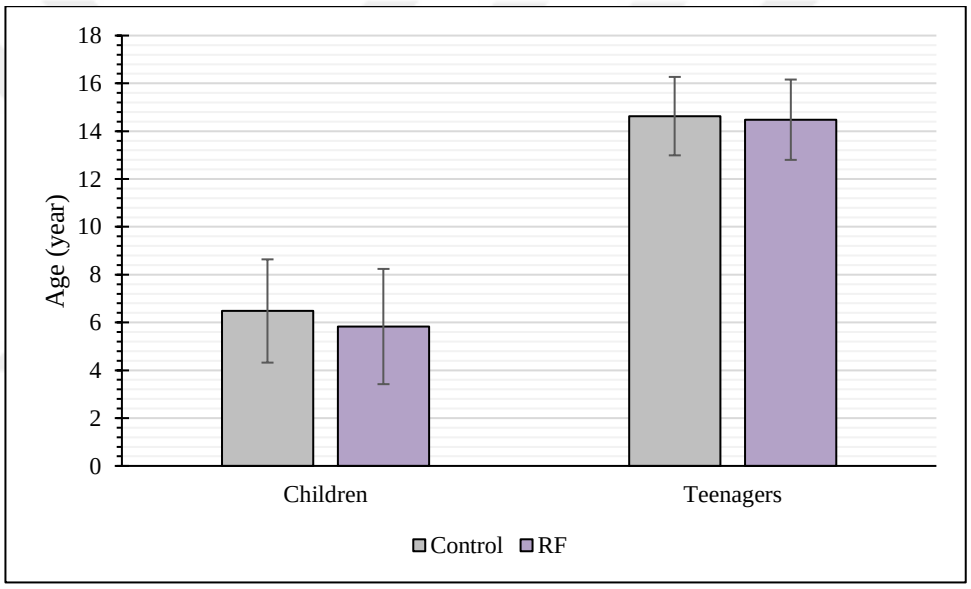


Figure 4.1 The age of children and teenagers with and without RF

4.2 Apelin

Apelin concentration in the sera of children with RF (281.41±96.34 pg/mL) was non-significant (p=0.996) in the statistics comparison with control children without RF (276.91±94.34 pg/mL). Additionally, the concentration of apelin was also non-significant (p=0.806) between teenagers with RF (263.28±90.81 pg/mL) and control teenagers without RF (281.97±90.82 pg/mL). Furthermore, the statistics have shown non-significant change between teenagers and control in apelin concentrations

neither in control nor in RF patients. The graphic illustration of data is seen in Figure 4.2.

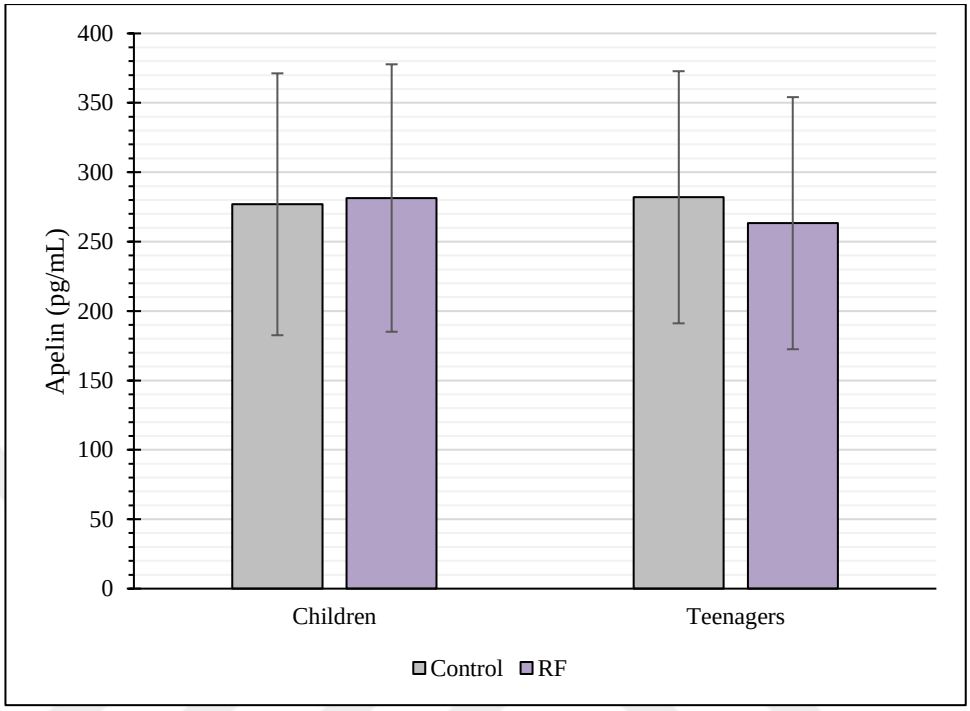


Figure 4.2 The concentration of apelin of children and teenagers with and without RF

4.3 Vitamin D

Vitamin D concentration in the sera of children with RF (24.65±6.91 ng/mL) was in significant reduction (p=0.0001) according to the statistics comparison with control children without RF (31.72±5.03 ng/mL). Additionally, the concentration of vitamin D was also in significant reduction (p=0.0001) in the sera of teenagers with RF (21.58±6.37 ng/mL) in comparison to control teenagers without RF (29.84±4.45 ng/mL). Furthermore, the statistics have shown non-significant change between teenagers and control in vitamin D concentrations neither in control nor in RF patients. The graphic illustration of data is seen in Figure 4.3.

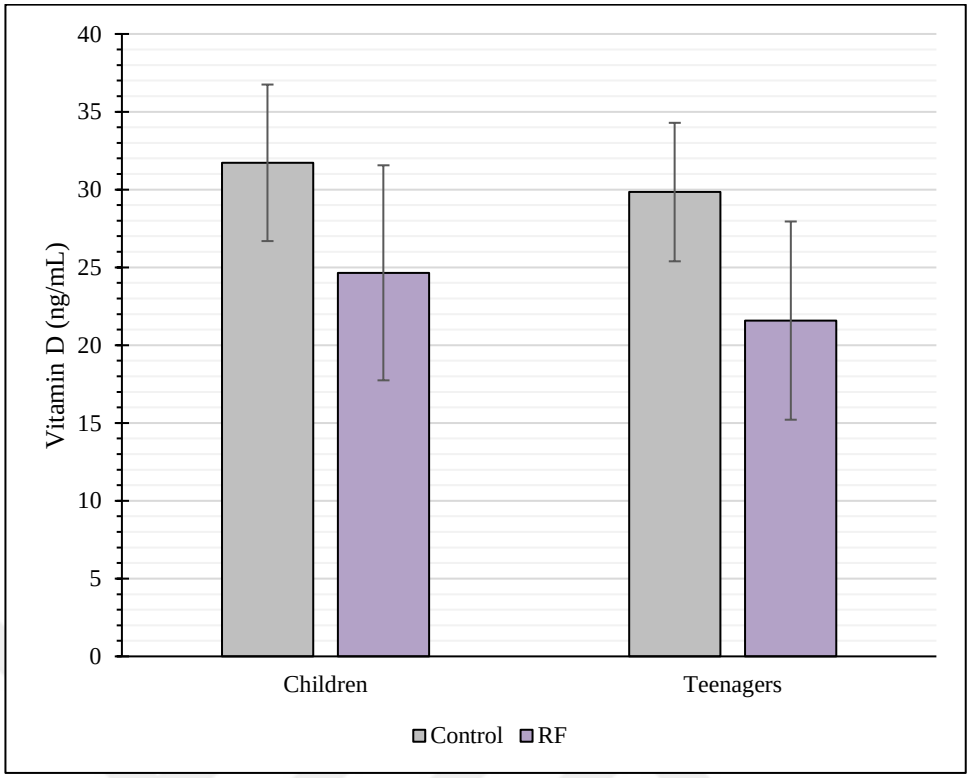


Figure 4.3 The concentration of vitamin D in the sera of children and teenagers with and without RF

4.4 Fasting Blood Sugar

The FBS concentration in the sera of children with RF (82.30±7.75 mg/dL) was non-significant (p=0.989) in the statistics comparison with control children without RF (81.75±7.64 mg/dL). Additionally, the concentration of FBS was also non-significant (p=0.529) between teenagers with RF (85.05±8.32 mg/dL) and control teenagers without RF (82.70±7.25 mg/dL). Furthermore, the statistics have shown non-significant change between teenagers and control in FBS concentrations neither in control nor in RF patients. The graphic illustration of data is seen in Figure 4.4.

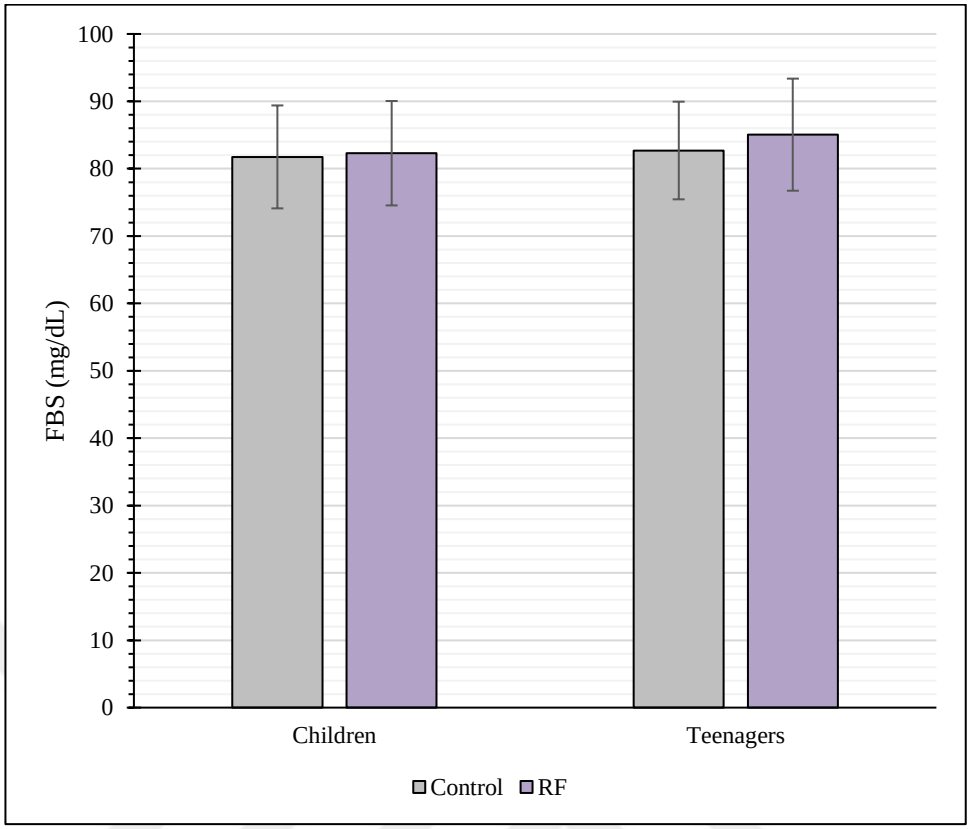


Figure 4.4 The concentration of FBS in the sera of children and teenagers with and without RF

4.5 Glycated Hemoglobin

The HbA1c concentration in the blood of children with RF (4.53±0.28 %) was non-significant (p=0.741) in the statistics comparison with control children without RF (4.46±0.27 %). Additionally, the concentration of HbA1c was also non-significant (p=0.962) between teenagers with RF (4.64±0.28 %) and control teenagers without RF (4.61±0.27 %). Furthermore, the statistics have shown non-significant change between teenagers and control in HbA1c concentrations neither in control nor in RF patients. The graphic illustration of data is seen in Figure 4.5.

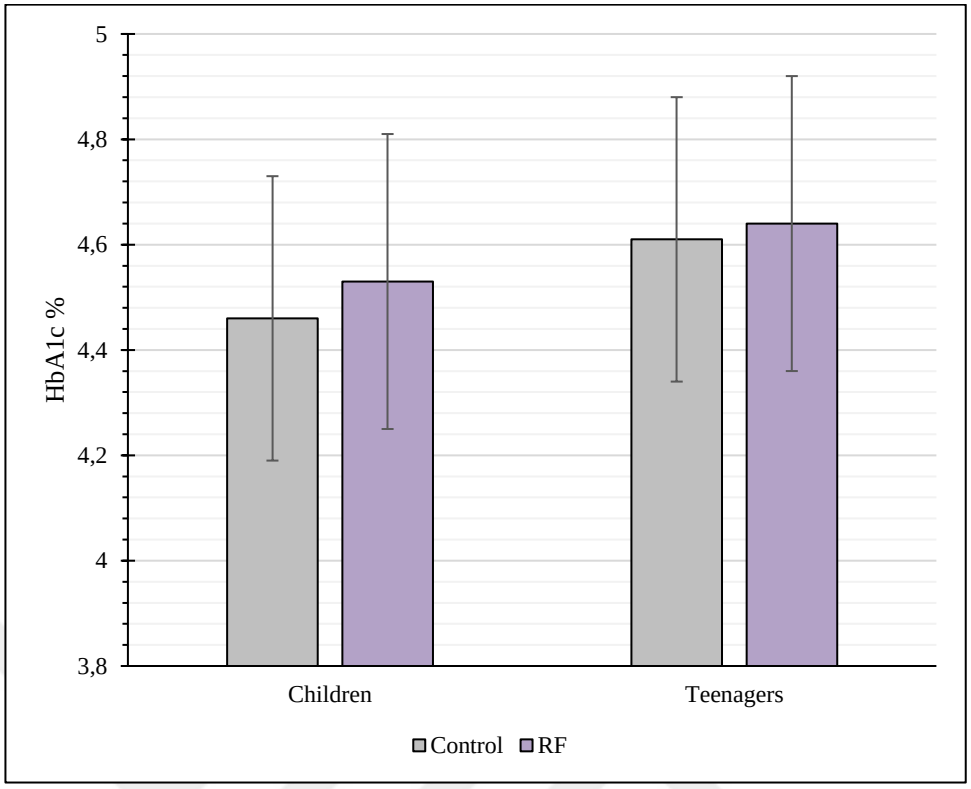


Figure 4.5 The concentration of HbA1c in the blood of children and teenagers with and without RF

4.6 Blood Urea

Urea concentration in the sera of children with RF (73.27±12.23 mg/dL) was in significant increase (p=0.0001) according to the statistics comparison with control children without RF (16.61±3.17 mg/dL). Additionally, the concentration of urea was also in significant increase (p=0.0001) in the sera of teenagers with RF (88.72±19.72 mg/dL) in comparison to control teenagers without RF (19.84±3.33 mg/dL). Furthermore, the statistics have shown non-significant change between teenagers and children in urea concentrations in control but significant (p=0.0001) higher urea concentration in RF teenagers compared to RF children. The graphic illustration of data is seen in Figure 4.6.

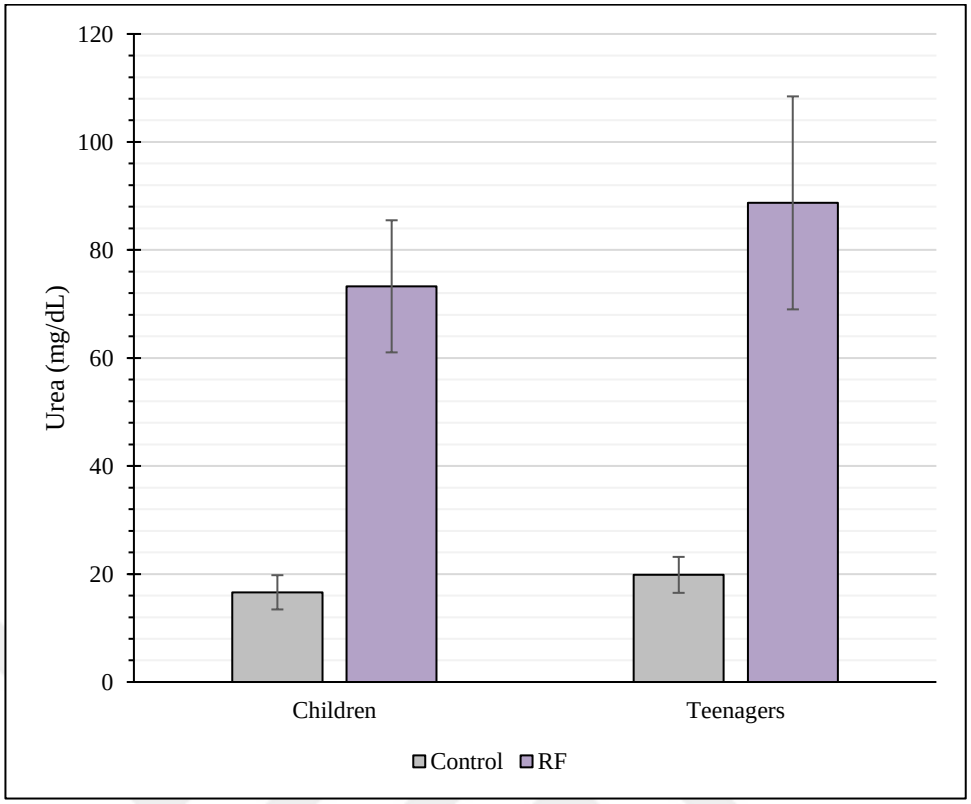


Figure 4.6 The concentration of urea in the sera of children and teenagers with and without RF

4.7 Serum Creatinine

Creatinine concentration in the sera of children with RF (2.65±1.01 mg/dL) was in significant increase (p=0.0001) according to the statistics comparison with control children without RF (0.57±0.11 mg/dL). Additionally, the concentration of creatinine was also in significant increase (p=0.0001) in the sera of teenagers with RF (2.72±1.13 mg/dL) in comparison to control teenagers without RF (0.70±0.17 mg/dL). Furthermore, the statistics have shown non-significant change between teenagers and control in creatinine concentrations neither in control nor in RF patients. The graphic illustration of data is seen in Figure 4.7.

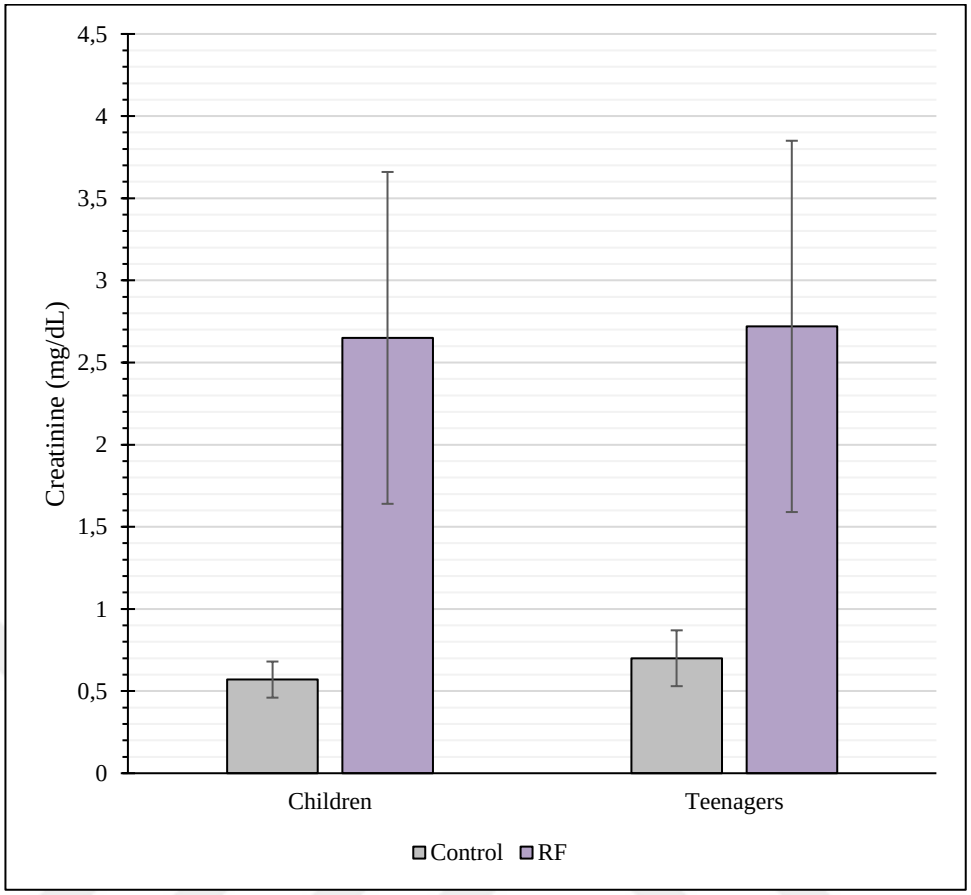


Figure 4.7 The concentration of creatinine in the sera of children and teenagers with and without RF

4.8 Cholesterol

Cholesterol concentration in the sera of children with RF (112.07±17.11 mg/dL) was in significant increase (p=0.023) according to the statistics comparison with control children without RF (103.91±13.45 mg/dL). But, the concentration of cholesterol was not significant (p=0.839) in the sera of teenagers with RF (118.32±21.53 mg/dL) in comparison to control teenagers without RF (115.09±16.43 mg/dL). Furthermore, the statistics have shown non-significant change between teenagers and control in cholesterol concentrations neither in control nor in RF patients. The graphic illustration of data is seen in Figure 4.8.

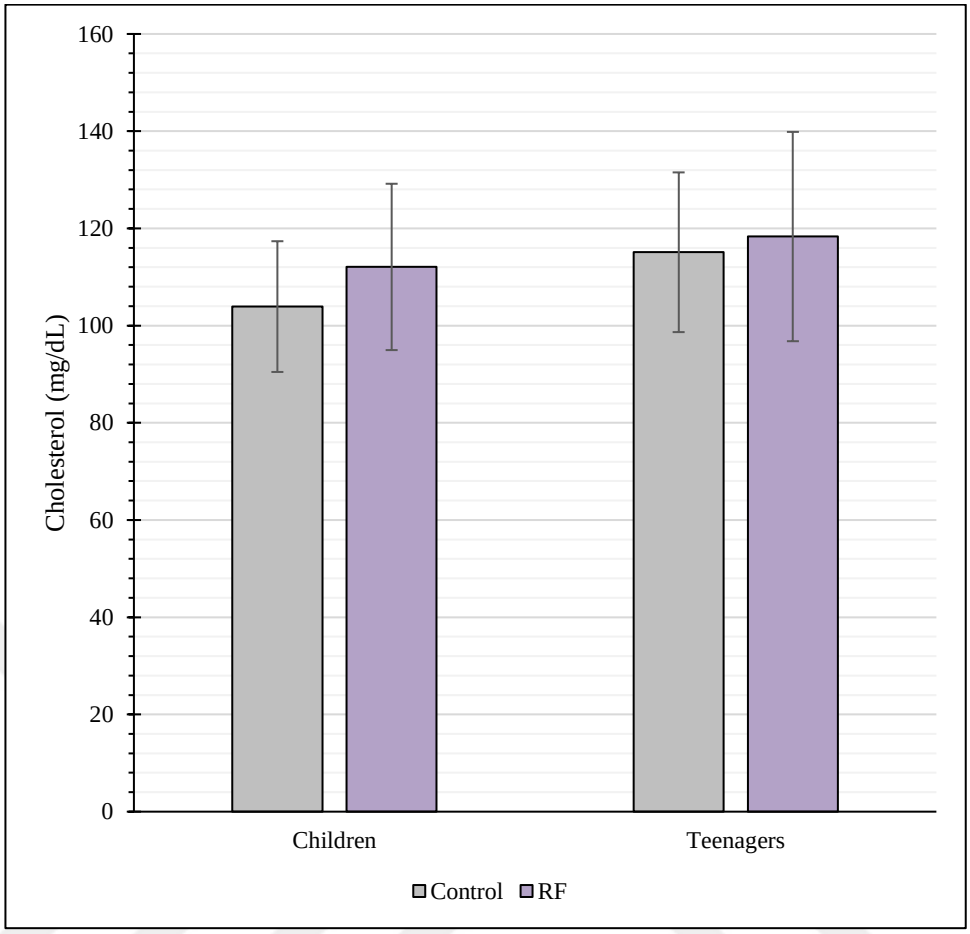


Figure 4.8 The concentration of cholesterol in the sera of children and teenagers with and without RF

4.9 Triacylglycerol

The triacylglycerol concentration in the sera of children with RF (69.79±5.16 mg/dL) was non-significant (p=0.701) in the statistics comparison with control children without RF (68.36±4.37 mg/dL). Additionally, the concentration of triacylglycerol was also non-significant (p=0.301) between teenagers with RF (88.41±6.54 mg/dL) and control teenagers without RF (86.10±7.16 mg/dL). Furthermore, the statistics have shown significant higher concentrations of triacylglycerol in teenagers and control in both control and RF patients. The graphic illustration of data is seen in Figure 4.9.

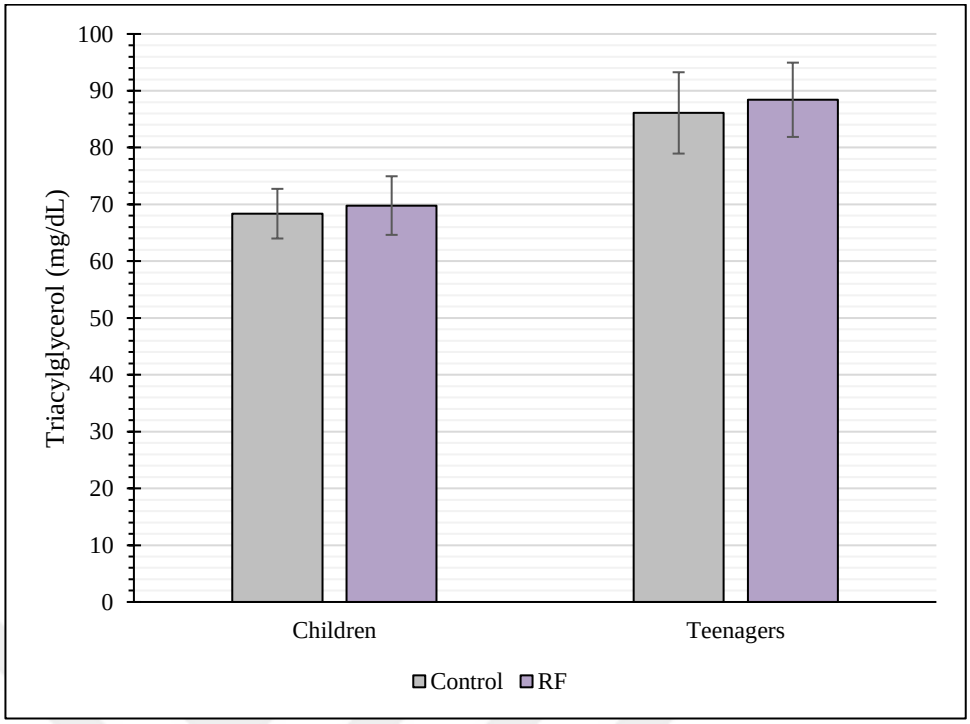


Figure 4.9 The concentration of triacylglycerol in the sera of children and teenagers with and without RF

4.10 High-Density Lipoprotein

The HDL concentration in the sera of children with RF (46.17±3.01 mg/dL) was in significant reduction (p=0.0001) according to the statistics comparison with control children without RF (50.03±4.65 mg/dL). Additionally, the concentration of HDL was also in significant reduction (p=0.0001) in the sera of teenagers with RF (45.03±3.66 mg/dL) in comparison to control teenagers without RF (50.68±5.28 mg/dL). Furthermore, the statistics have shown non-significant change between teenagers and control in HDL concentrations neither in control nor in RF patients. The graphic illustration of data is seen in Figure 4.10.

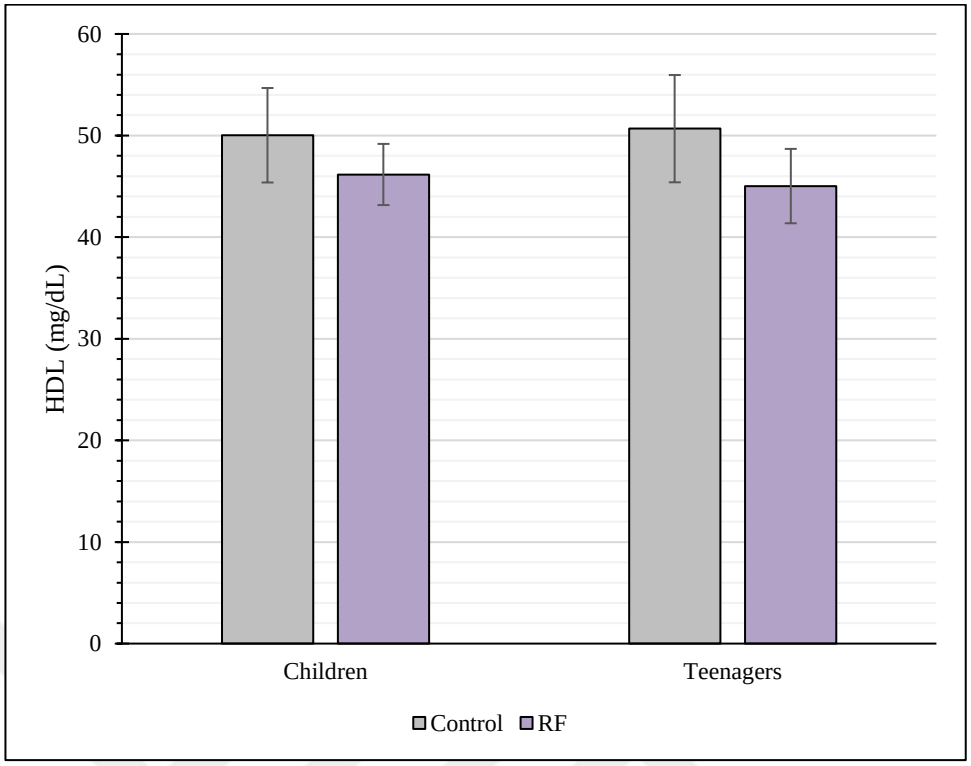


Figure 4.10 The concentration of HDL in the sera of children and teenagers with and without RF

4.11 Low-Density Lipoprotein

The LDL concentration in the sera of children with RF (51.94±17.42 mg/dL) was in significant increase (p=0.008) according to the statistics comparison with control children without RF (40.21±10.54 mg/dL). But, the concentration of LDL was not significant (p=0.095) in the sera of teenagers with RF (55.61±20.83 mg/dL) in comparison to control teenagers without RF (47.19±13.92 mg/dL). Furthermore, the statistics have shown non-significant change between teenagers and control in LDL concentrations neither in control nor in RF patients. The graphic illustration of data is seen in Figure 4.11.

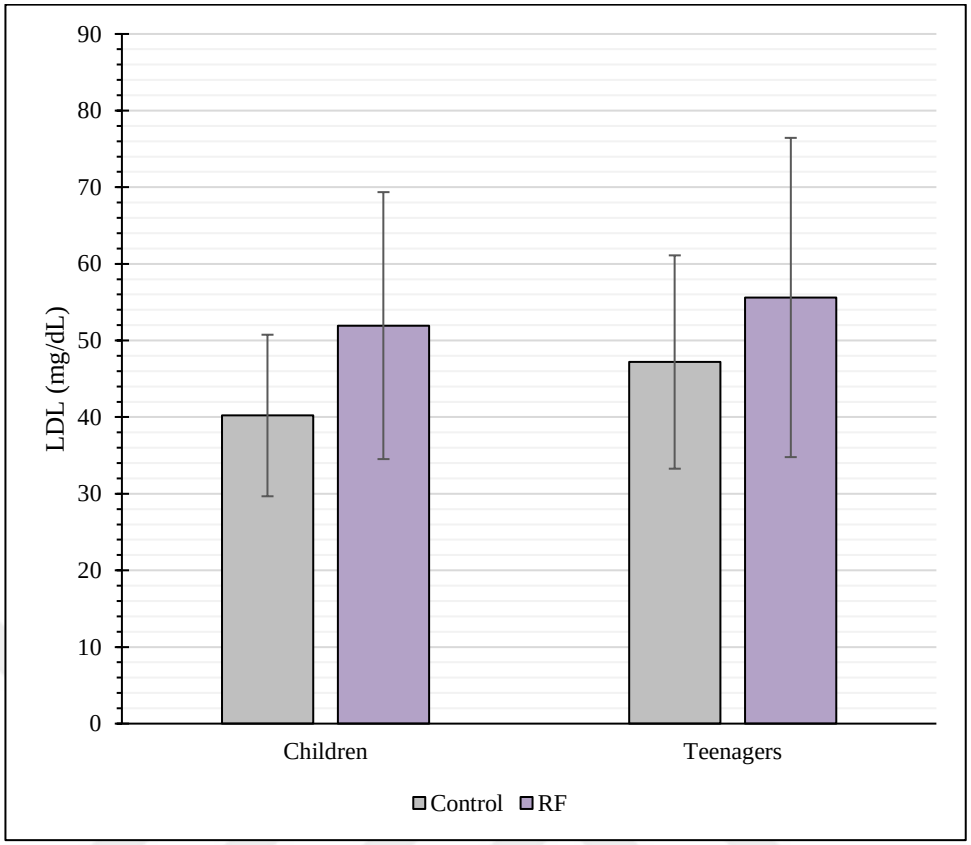


Figure 4.11 The concentration of LDL in the sera of children and teenagers with and without RF

4.12 Very Low-Density Lipoprotein

The VLDL concentration in the sera of children with RF (13.96±1.03 mg/dL) was non-significant (p=0.701) in the statistics comparison with control children without RF (13.67±0.87 mg/dL). Additionally, the concentration of VLDL was also non-significant (p=0.301) between teenagers with RF (17.68±1.31 mg/dL) and control teenagers without RF (17.22±1.43 mg/dL). Furthermore, the statistics have shown significant higher concentrations of VLDL in teenagers and control in both control and RF patients. The graphic illustration of data is seen in Figure 4.12.

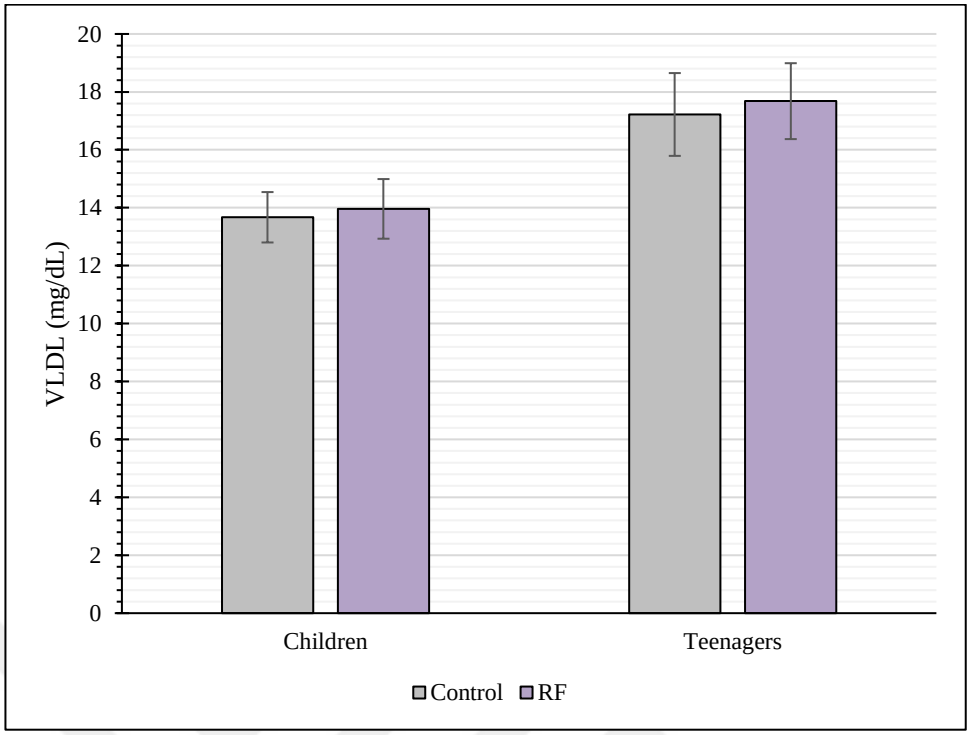


Figure 4.12 The concentration of LDL in the sera of children and teenagers with and without RF

4.13 Arginase

Arginase concentration in the sera of children with RF (2.36±1.06 ng/mL) was in significant increase (p=0.0001) according to the statistics comparison with control children without RF (1.04±0.37 ng/mL). Additionally, the concentration of arginase was also in significant increase (p=0.0001) in the sera of teenagers with RF (3.01±1.46 ng/mL) in comparison to control teenagers without RF (1.09±0.42 ng/mL). Furthermore, the statistics have shown non-significant change between teenagers and children in arginase concentrations in control but significant (p=0.013) higher arginase concentration in RF teenagers compared to RF children. The graphic illustration of data is seen in Figure 4.13.

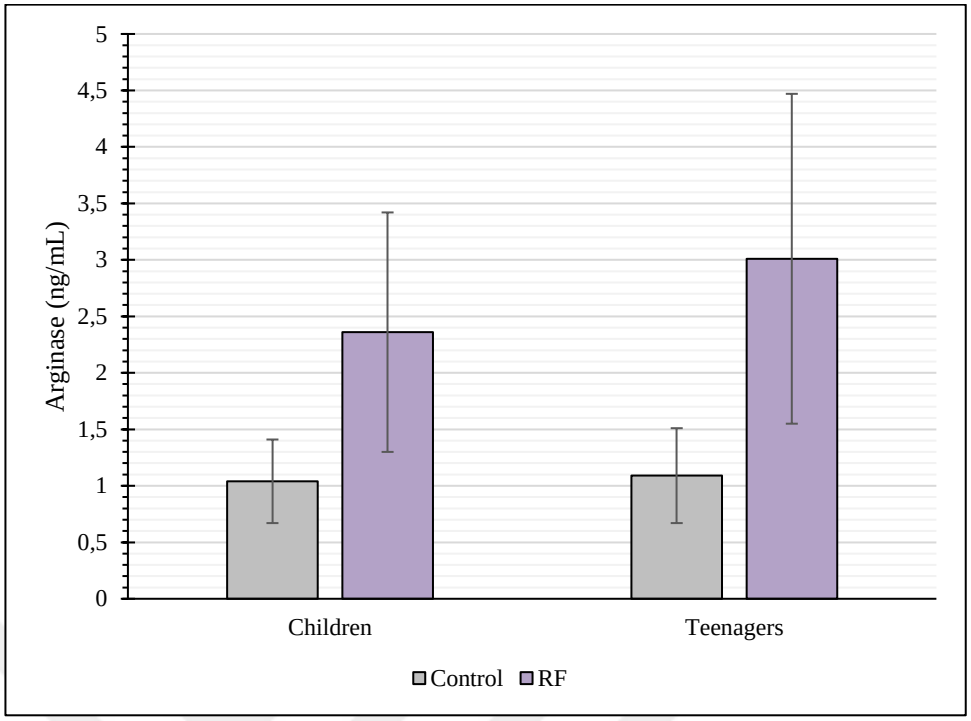


Figure 4.13 The concentration of arginase in the sera of children and teenagers with and without RF

Table 4.1 List the mean ± SD of the results of this study

Parameters	**Mean±SD**			
	Control children without RF	Children with RF	Control teenagers without RF	Teenagers with RF
age	6.48±2.16	5.83±2.41	14.63±1.64	14.48±1.68
Vitamin D	31.72±5.03	24.65±6.91	29.84±4.45	21.58±6.37
Apelin	276.91±94.34	281.41±96.34	281.97±90.82	263.28±90.81
Arginase	1.04±0.37	2.36±1.06	1.09±0.42	3.01±1.46
VLDL	13.67±0.87	13.96±1.03	17.22±1.43	17.68±1.31
Creatinine	0.57±0.11	2.65±1.01	0.70±0.17	2.72±1.13
LDL	40.21±10.54	51.94±17.42	47.19±13.92	55.61±20.83
Urea	16.61±3.17	73.27±12.23	19.84±3.33	88.72±19.72
HDL	50.03±4.65	46.17±3.01	50.68±5.28	45.03±3.66
HbA1c	4.46±0.27	4.53±0.28	4.61±0.27	4.64±0.28
triacylglycerol	68.36±4.37	69.79±5.16	86.10±7.16	88.41±6.54
FBS	81.75±7.64	82.30±7.75	82.70±7.25	85.05±8.32
Cholesterol	103.91±13.45	112.07±17.11	115.09±16.43	118.32±21.53

4.14 Correlation in Children with RF

The studied variables were tested for their correlation (apelin, vitamin D, and arginase) in children with RF disease, and the collected data are shown in Table 4.1.

Table 4.2 Correlation of apelin, vitamin D, and arginase in children with RF disease

VARIABLE	APELIN		VITAMIN D		ARGINASE	
	r	*p*-value	r	*p*-value	r	*p*-value
Vitamin D	-0.059	0.718	-	-	-0.436	0.005
Arginase	-0.137	0.398	-0.436	0.005	-	-
Age	-0.135	0.407	0.177	0.275	0.033	0.840
FBS	-0.013	0.937	-0.094	0.566	0.093	0.569
HbA1c	0.040	0.805	-0.095	0.562	0.196	0.225
Urea	-0.168	0.299	0.169	0.297	-0.071	0.662
Creatinine	0.161	0.322	-0.107	0.511	-0.191	0.238
Cholesterol	0.043	0.790	-0.277	0.084	-0.024	0.881
Triacylglycerol	-0.093	0.567	-0.154	0.342	0.213	0.188
HDL	0.170	0.295	0.447	0.004	-0.330	0.038
LDL	0.019	0.908	-0.340	0.032	0.020	0.901
VLDL	-0.093	0.567	-0.154	0.342	0.213	0.188

The children who have RF disease showed a correlation between vitamin D from a side, arginase (negative and weak; Figure 4.14), HDL (positive and weak; Figure 4.15), and LDL (negative and weak; Figure 4.16) from the other side.

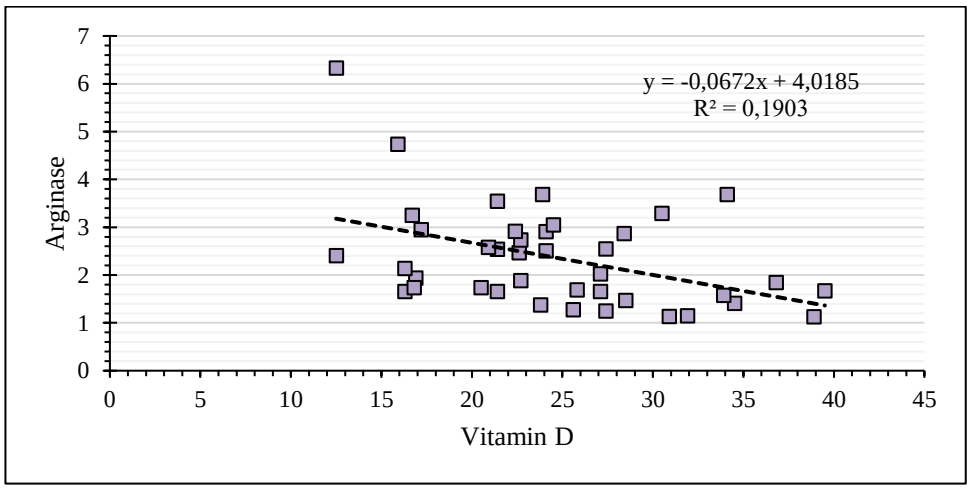


Figure 4.14 Correlation between vitamin D and arginase in children with RF

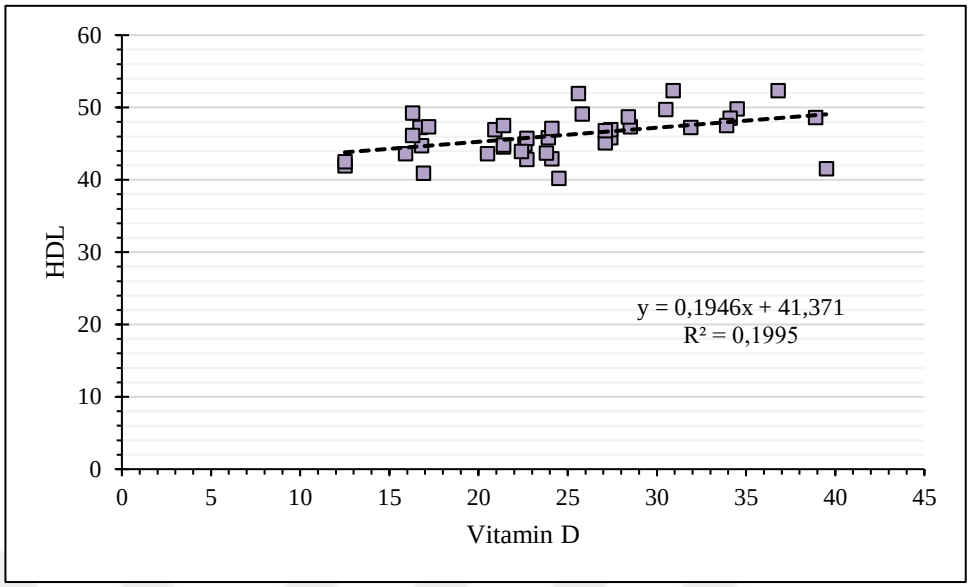


Figure 4.15 Correlation between vitamin D and HDL in children with RF

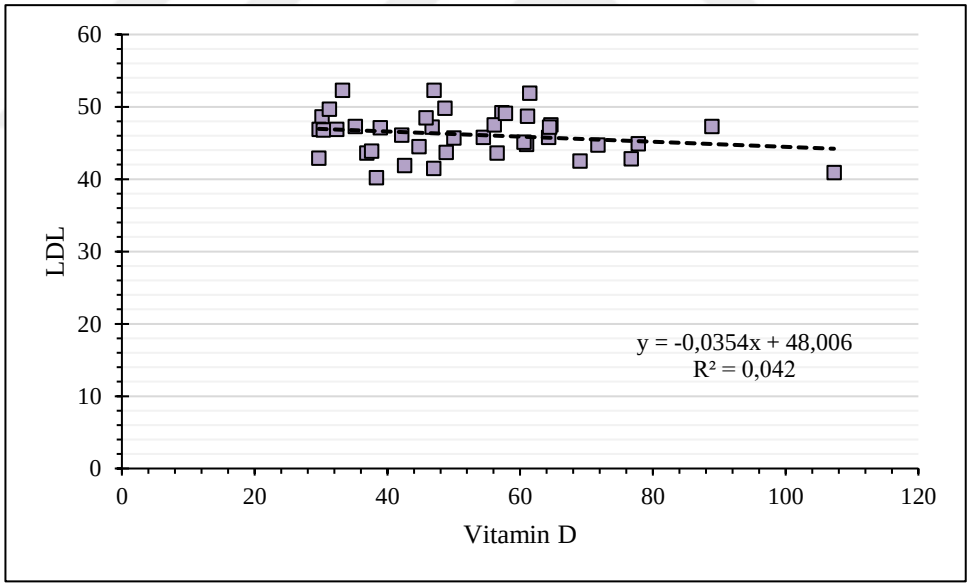


Figure 4.16 Correlation between vitamin D and LDL in children with RF

One more, the children with RF disease were showed a negative correlation between arginase and HDL, which graphed in Figure 4.17.

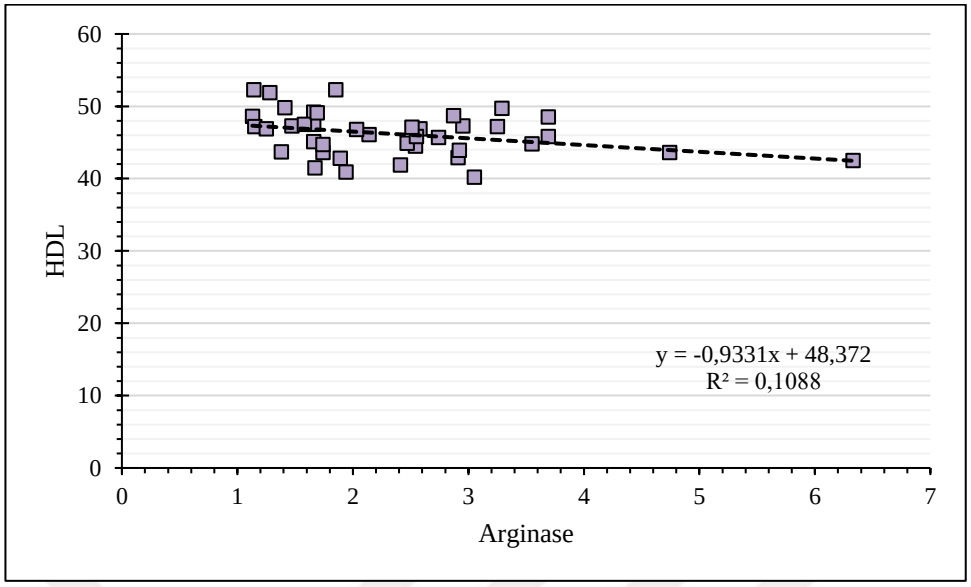


Figure 4.17 Correlation between arginase and HDL in children with RF

4.15 Correlation in Teenagers with RF

The studied variables were tested for their correlation (apelin, vitamin D, and arginase) in teenagers with RF disease, and the collected data are shown in Table 4.2. The data have revealed no significant association between the variables in teenagers with RF disease.

Table 4.3 Correlation of apelin, vitamin D, and arginase in teenagers with RF disease

VARIABLE	APELIN		VITAMIN D		ARGINASE	
	r	*p*-value	r	*p*-value	r	*p*-value
Vitamin D	-0.215	0.183	-	-	0.005	0.978
Arginase	-0.184	0.255	0.005	0.978	-	-
Age	-0.022	0.892	0.236	0.142	0.013	0.937
FBS	-0.009	0.956	0.087	0.594	0.060	0.715
HbA1c	-0.215	0.182	-0.015	0.926	-0.001	0.997
Urea	0.164	0.312	0.288	0.072	0.099	0.544
Creatinine	-0.036	0.826	0.205	0.203	-0.274	0.087
Cholesterol	-0.128	0.430	0.200	0.2017	-0.092	0.574
Triacylglycerol	0.152	0.350	-0.107	0.511	-0.220	0.173
HDL	0.116	0.476	0.213	0.186	-0.065	0.691
LDL	-0.163	0.316	0.176	0.278	-0.069	0.670
VLDL	0.152	0.350	-0.107	0.511	-0.220	0.173

5. DISCUSSION

At the current research we attempt to study the change in apelin, vitamin D, and arginase as principle biomarkers in children and teenagers with RF disease. Two groups of control were enrolled according to their age to get an optimum comparison strategy, leaving the effect of age (especially the changes that happen during puberty) out of the study.

Yavuz *et al.* have shown in their study that apelin was not differed in children on dialysis, and they have clarify that apelin was not useful in monitoring RF in children (Yavuz *et al.* 2014), this observation was agreed with our research data. Lu *et al.* have reported no remarkable change of apelin concentration in patients with chronic disease of the kidneys at any pathological stage, with each other and with individuals without any problems in the kidneys (Lu *et al.* 2020), which was also agreed with our research data. Cernaro *et al.* have revealed that apelin does not change remarkably in patients on hemodialysis beyond the normal range of apelin in the circulation (Cernaro *et al.* 2012).

Nevertheless, Lacquaniti *et al.* study has shown systemic decrease of apelin concentration in individuals with polycystic kidney problems (Lacquaniti *et al.* 2013) which was reported in similar observations in later study (Kocer *et al.* 2016), while Szczepańska *et al.* study has shown systemic increase of apelin concentration in individuals at the fifth stage of chronic kidney disease (Szczepańska *et al.* 2015).

In the work of Holick, the author have reviewed a studies regarding vitamin D before the year of 2005, and he was found that a deficiency in vitamin D could increase the risks of renal impairment in children and adults. Moreover, the author reported a link between the circulation vitamin D levels and hyperoarathyroidism in patients with CKD (Holick, 2005). Hari *et al.* have reported that a reduction of vitamin D circulation level is frequent in individuals with kidneys problems, and they have related this reduction to the health of bones in these patients. The authors have observed a mean of vitamin D circulation level below 20 ng/mL in these patients, and they have shown that the reduction of vitamin D was unrelated to the stage of the disease (Hari *et al.* 2010).

Belostotsky *et al.* have found that 32% of children with renal failure in their study were have circulation vitamin D below 25 ng/mL, and this reduction was greatly linked with the level of parathyroid hormone in these children (Belostotsky *et al.* 2008). Shroff *et al.* have collected a previous documents and trials on using vitamin D in the treatment of children with different stages of chronic kidney disease. The authors have shown that a supplementation with vitamin D for children reduces the health risks and restore the normal bone metabolism (Shroff *et al.* 2017).

Arginase is an enzyme related to the production of urea in the physiological machinery of the human body (Caldwell *et al.* 2015). In the work of Kayali *et al.* arginase level was greatly increased in individuals with renal dysfunction (Kayali *et al.* 2009). In another study, it was reported that inhibition of arginase enzyme showed a beneficial effects in the diabetic mouse with kidneys (You *et al.* 2013). Eleftheriadis *et al.* have reported that the increase of arginase I in renal failure patients on hemodialysis could indicate an ischemic heart disease, while those without any heart problems do not exhibit important change of arginase I (Eleftheriadis *et al.* 2011). Yiannaki *et al.* on the other hand, reported an important increase of arginase I in RF patients (Eleftheriadis *et al.* 2012).

Children and teenagers with RF disease in our study have shown normal outcomes of FBS, HbA1c, triacylglycerol, and VLDL. Cholesterol was increased in children with RF disease, while HDL was decreased in both children and teenagers with RF disease. A previous study have shown a remarkable change of cholesterol, triacylglycerol and HDL in children with RF disease (Ragab and Ragab, 2004), which agreed with our observations.

6. CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

1. Apelin was not changed in children and teenagers with RF disease when compared to control without RF disease at similar age.
2. Vitamin D was reduced significantly in the sera of children and teenagers with RF disease when compared to control without RF disease at similar age.
3. Arginase concentration was observed to be increased significantly in children and teenagers with RF disease.
4. Cholesterol and LDL were increased in children, but not teenagers, with RF disease. HDL was decreased in both children and teenagers with RF disease, while triacylglycerol and VLDL were not changed.
5. FBS and HbA1c were normal in children and teenagers with RF disease.
6. Children who have RF disease were showed positive correlation between vitamin D and HDL, and negative correlation between vitamin D, and LDL, and arginase.
7. Children who have RF disease were showed negative correlation between arginase and HDL.

6.2 Recommendations

1. Targeting arginase in the treatment of RF disease in children and teenagers.
2. Using vitamin D supplements as part of therapeutic approach in the treatment of RF disease in children and teenagers.
3. Evaluation of apelin level in the urine of children and adults with RF disease.
4. Estimation of nitric oxide synthase activity in children and adults with RF disease.

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