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# DETERMINATION OF THE RELATIONSHIP BETWEEN DNA METHYLATION STATUS OF *KLOTHO* AND *ARNTL* GENES WITH HYPERTENSION

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## ABSTRACT

Hypertension is a multifactorial chronic disease due to the interaction of environmental factors with genetic alteration. *KLOTHO* and *ARNTL* genes play an important role in the development of hypertension. Therefore, we analyzed the methylation status of *KLOTHO* and *ARNTL* genes by using methylation-sensitive high-resolution melting (MS-HRM) in a total of 78 hypertensive and 49 control subjects. In this study, we could not identify a significant association between *KLOTHO* and *ARNTL* methylation and the hypertensive phenotype. Moreover, we could not find a direct association between *KLOTHO* and *ARNTL* methylation and the fasting blood sugar, triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, sodium (Na), creatinine (Cr), potassium (K), and urea levels in hypertensive patients. However, we found a significant difference between the methylated *KLOTHO* hypertensive patients and the unmethylated *KLOTHO* control subjects for potassium (K).

***Keywords:*** Methylation, Hypertension, *KLOTHO*, *ARNTL*

## INTRODUCTION

Hypertension (HTN) is a common chronic disease in advanced age and is considered to influence 29% of adult individuals worldwide by 2025 [1]. HTN is one of the leading causes of kidney disease and cardiovascular complications, including stroke, coronary artery disease, heart failure, and peripheral vascular disease [2]. Generally, HTN can be categorized into two groups, as primary (essential or idiopathic) and secondary (non-essential) hypertension. Approximately 90-95% of hypertensive cases have primary hypertension, 5-10% of hypertensive cases have secondary hypertension. Primary hypertension is not due to other diseases and is affected by genetic and lifestyle factors. However, secondary hypertension is developed from kidney diseases, endocrine disorders, or side effects of drugs [3]. Mechanisms involved in the development of primary hypertension have been reported to be still unclear [1]. Numerous factors, including genetic, epigenetic, advanced age, smoking, overweight, diabetes, arterial aging, endothelial dysfunction, and arteriosclerosis contribute to HTN [4,5]. The heritability for HTN was indicated to be 30-60% [6].

*KLOTHO* (*KL*) was discovered as an aging suppressor gene encoding α-KL protein due to the determination of short lifespan and various aging-related phenotypes resembling human aging. These phenotypes include vascular calcification, atherosclerosis, cardiovascular disease in *KL*-deficient mice [7]. The *KL* gene is highly conserved in mice, rats and humans [8,9], and mainly expressed in the distal convoluted and proximal tubules of the kidney, choroid plexus of the brain and also other tissues such as the parathyroid glands, sinoatrial node, vascular tissue, cartilage and bones [10]. More than 10 SNPs in the human *KL* gene have been reported [11,12]. *KL* gene polymorphisms such as G-395A [5,13], C1818T [2], and F352V [14] were associated with hypertension. The *KL* gene expression was shown to be lower in essential hypertensive patients in the Indian population [3]. Furthermore, a decrease in *KL* levels has been found in renovascular hypertensive

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patients. Thus, these results shed light on how the *KL* gene plays a crucial role in the pathophysiology of primary and secondary hypertension. Additionally, according to these results, it was suggested that *KL* could be utilized as a biomarker for the determination of kidney injury in hypertensive patients [1,15].

The circadian clock is a highly conserved system and provides adaptations of organisms to the environment cues along the 24-hour light/dark cycle [16]. It regulates most physiological processes, such as sleep-wake cycles, immune response, metabolism, renal function, and blood pressure [17]. The circadian clock is involved in the maintenance of vascular function. It has been suggested that impaired clock function can lead to health complications such as cardiovascular disease and hypertension by contributing to vascular dysfunction [16, 18]. Aryl hydrocarbon receptor nuclear translocator-like protein 1 (*ARNTL* or brain and muscle ARNT-like 1 (*BMAL1*)) is a core clock component and regulates the rhythmic expression of many genes as a transcription factor [19]. The *ARNTL* rs9633835 and rs6486121 polymorphisms were associated with susceptibility to hypertension [20,21].

There have been studies investigating the connection between the *KL* and *ARNTL* genes and hypertension through polymorphism and expression analyses, there is no published study that explores the correlation between the methylation status of these genes and hypertension, as well as the associated biochemical variables in both hypertensive and control subjects. Methylation is an epigenetic modification process that alters gene expression without changing the nucleotide sequence, thus contributing to the regulation of gene expression and maintenance of genomic stability[22]. In this study, therefore, the methylation status of the *KL* and *ARNTL* genes was analyzed in both hypertensive and control subjects. Furthermore, the possible effect of *KL* and *ARNTL* gene methylation on biochemical variables was analyzed in hypertensive and control subjects.

## MATERIAL AND METHODS

**Study subjects**

78 hypertensive patients (31 female and 47 male) and 49 control subjects (36 female and 13 male) were included in this study, and peripheral blood specimens of the participants were collected. Participants were recruited from Burhan Nalbantoğlu State Hospital, Nicosia (from November 2022 to April 2023). The diagnostic criteria of hypertension were defined as systolic blood pressure (SBP)/diastolic blood pressure (DBP) >140/90 mmHg or antihypertensive medication use for decreasing high blood pressure [23]. The participants with cancer, respiratory diseases, cerebral infarction, congenital heart disease, diabetes mellitus or chronic kidney diseases, and autoimmune diseases were excluded, moreover, subjects who were discontent to participate in the study were excluded as well. The medical history of all participants was questioned. All clinical investigations performed for this study were conducted in accordance with the principles of the Declaration of Helsinki. The study was approved by the Scientific Research Ethics Committee of the Near East University (YDU/2020/80-1066). All subjects signed the written consent form before participating in the study.

**Measurements of the Biochemical Parameters**

Peripheral blood samples were collected from participants after overnight fasting. All participants' serum was obtained by centrifugation at 2000 rpm for 20 min at 4 °C. The fasting blood sugar (FBS), triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), sodium (Na), potassium (K), creatinine (Cr), and urea levels were determined with an automated clinical biochemistry analyzer (Abbott Architect C8000).

**Methylation Analysis**

Peripheral blood samples of hypertensive patients and normal controls were transferred to 2 ml vacuum tubes with K2EDTA. Genomic DNA was isolated from whole blood samples by using the AllPrep DNA/RNA/Protein isolation kit (Qiagen in Manchester, United Kingdom). A NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA USA) was used to analyze the amount and purification of DNA samples. Sodium bisulfite modification of DNA samples was done by using the EpiTect Bisulfite Modification Kit (Qiagen, Manchester, UK). According to the EpiTect® MS-HRM PCR Handbook (Qiagen, Manchester, UK), primers were designed. The MS-HRM analysis was performed to analyze the promotor methylation status of *KLOTHO* and *ARNTL* genes according to the EpiTect® HRM™ PCR Handbook protocol (Rotor-Gene Q, Qiagen). As methylated and unmethylated controls, universal methylated and unmethylated DNA (EpiTect Control DNA Set, Cat No./ID: 59568) were preferred [24].

**Statistical Analysis**

Descriptive statistics for qualitative (frequency and percentage) and quantitative varaibles (arithmetic mean, standard deviation) were calculated. Pearson's Chi-Square test or Fisher's exact test was used to investigate the associations between gene methylation status and several patient characteristics, where appropriate. The McNemar

test was used to investigate the association of methylation in both genes. A two-way Analysis of Variance test was performed to analyze the effects of gene methylation and hypertension on several biochemical parameters. Sidak's posthoc test was applied to investigate the pairwise differences, in the case of statistical significance. The Statistical Package for Social Science (SPSS) (Demo Version 26.0 for Mac) and GraphPad Prism (Demo Version 9.51 for Mac) software were used for all statistical calculations. The level of significance was accepted to be 0.05.

## RESULTS

The mean age of 76 patients who suffered from hypertension is 60.04 ± 13.57 years old, while the control subjects were at 44.45 ± 13.94 years old (n=49).

### Promoter methylation status of *KLOTHO* and *ARNTL* in hypertensive and control subjects

A comparison of the methylation status of *KLOTHO* and *ARNTL* in hypertensive and control subjects was shown in Table 1.

A total of 46 of the 67 methylated *ARNTL* subjects were hypertensive (68.7%) while 27 of the 48 unmethylated *ARNTL* subjects were hypertensive individuals (56.2%). The association between the methylation status of the *ARNTL* gene and hypertension was not significant ($p > 0.05$) (Table 1 and Figure 1-2).

A total of 62 of the 100 methylated *KLOTHO* subjects were hypertensive (62.0%). On the other hand, 16 of the 27 unmethylated *KLOTHO* subjects were hypertensive (59.3%). Likewise, for the *ARNTL* gene, the association between the methylation status of the *KLOTHO* gene and hypertension was not significant ($p > 0.05$) (Table 1 and Figure 3).

### The difference in metabolic characteristics between methylation levels of the *KLOTHO* and *ARNTL* genes

The mean values of biochemical parameters for *KLOTHO* and *ARNTL* genes regarding the methylation status were shown in Table 2 and Table 3, respectively.

The difference between the methylation status categories of *KLOTHO* and *ARNTL* genes for age, glucose, triglyceride, total cholesterol, HDL-C, and LDL-C levels in blood circulation, Na, K, Cr, Urea were investigated in this study. The results indicate no statistically significant difference for age, glucose, triglyceride, total cholesterol, HDL-C, and LDL-C levels in blood circulation, Na, K, Cr, Urea for both genes ($p > 0.05$).

**Table 1.** *KLOTHO* and *ARNTL* gene methylation in hypertensive and control subjects

| Subjects | *KLOTHO* gene | | | | *ARNTL* gene | | | |
|---|---|---|---|---|---|---|---|---|
| | Methylation | | Unmethylation | | Methylation | | Unmethylation | |
| | Count | $\%_A$ | Count | $\%_B$ | Count | $\%_A$ | Count | $\%_B$ |
| **Hypertension** | 62 | 62.0% | 16 | 59.3% | 46 | 68.7% | 27 | 56.2% |
| **Control** | 38 | 38.0% | 11 | 40.7% | 21 | 31.3% | 21 | 43.8% |
| **Total** | 100 | 100% | 27 | 100% | 67 | 100% | 48 | 100% |
| **p Value** | p>0.05 | | | | p>0.05 | | | |

(% in total according to methylation status in subjects for each gene)
($\%_A$ in total methylated subjects; $\%_B$ in total unmethylated subjects)

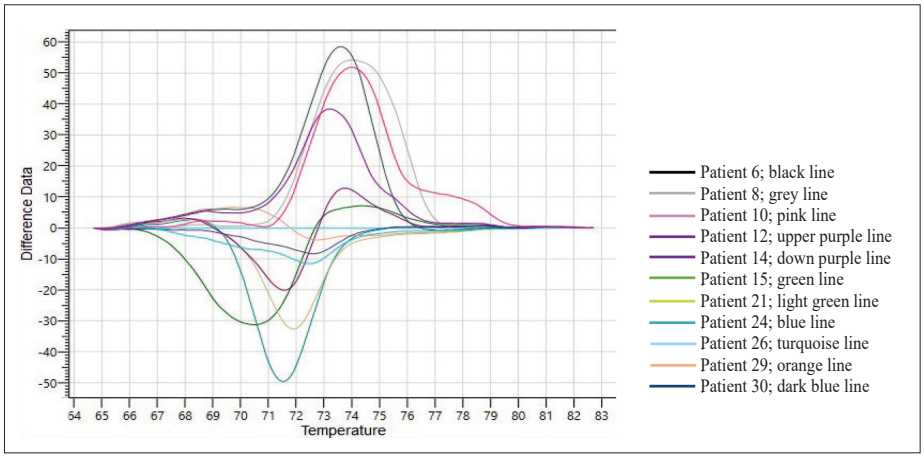


**Figure 1.** Methylated and unmethylated peaks of *ARNTL* gene for the patient groups. (The peaks obtained in different Tm degrees for the unmethylated and methylated cases were shown. Methylated *ARNTL* patient 6, 8, 10, and 12 are shown in black, grey, pink, and upper purple, respectively. Unmethylated *ARNTL* patient 14, 15, 21, 24, 26, 29, and 30 are shown in down purple, green, light green, blue, turquoise, orange, and dark blue, respectively)

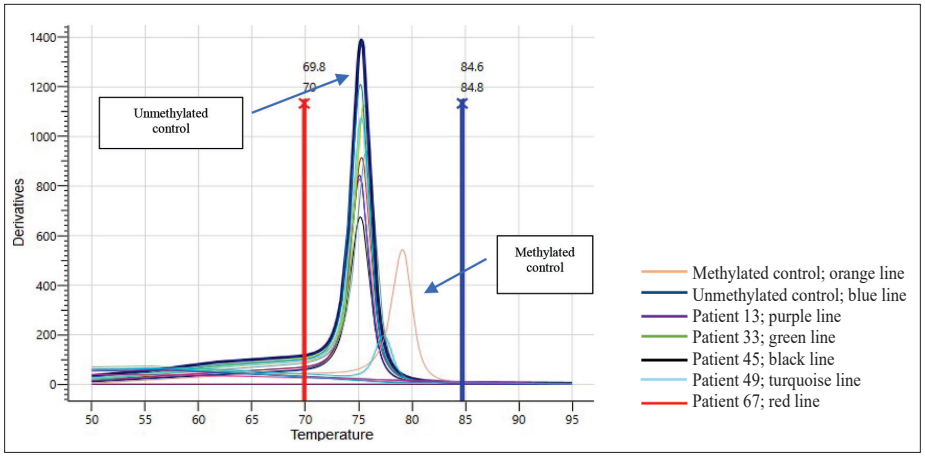


**Figure 2.** Unmethylated *ARNTL* patients. *ARNTL* unmethylated control is shown in blue, and methylated control is shown in orange. Unmethylated *ARNTL* patient 33, 49, 67, 13 and 45 are shown in green, turquoise, red, purple, and black, respectively.

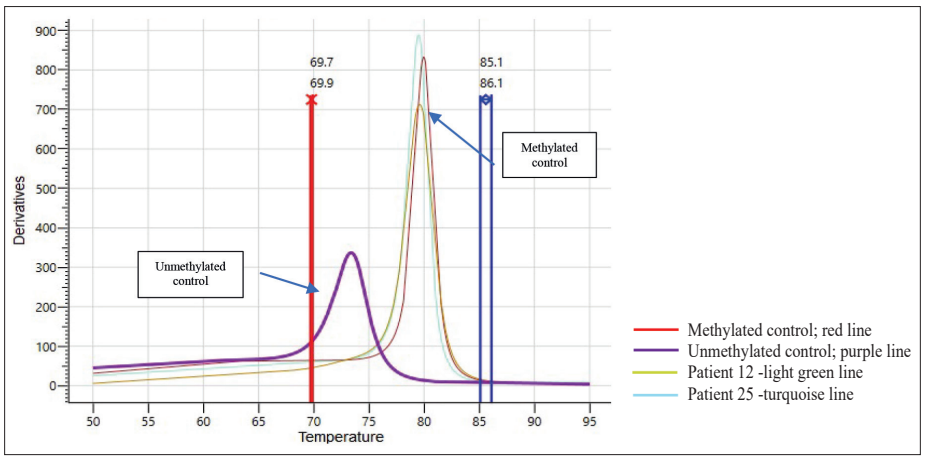


**Figure 3.** Methylated *KLOTHO* patients. *KLOTHO* unmethylated control is shown in purple, and methylated control is shown in red. Methylated *KLOTHO* patient 25 and 12 are shown in turquoise and light green, respectively.

**Table 2.** Mean Values of Biochemical Parameters for *KLOTHO* Gene

| Parameter | Methylated *KLOTHO* | | Unmethylated *KLOTHO* | |
|---|---|---|---|---|
| | Hypertensive subjects | Control Subjects | Hypertensive subjects | Control subjects |
| **Fasting glucose (mg/dL)** | 129.96 ± 83.08 (54) | 97.97 ± 12.43 (38) | 117.20 ± 34.03 (15) | 98.18 ± 5.23 (11) |
| **Triglyceride (mg/dL)** | 166.82 ±91.28 (38) | 122.22 ± 31.10 (37) | 183.93 ±104.06 (14) | 132.00±28.43 (11) |
| **Total cholesterol (mg/dL)** | 195.97 ± 61.63 (36) | 170.59 ±43.12 (37) | 197.07 ± 49.94 (14) | 191.09 ± 42.03 (11) |
| **HDL-C (mg/dL)** | 45.84 ± 14.31 (37) | 52.19 ± 12.72 (37) | 46.50 ± 14.34 (14) | 49.64 ± 6.83 (11) |
| **LDL-C (mg/dL)** | 131.92 ± 65.55 (37) | 129.62 ± 28.28 (37) | 125.71 ± 60.77 (14) | 130.27 ± 33.02 (11) |
| **Sodium (Na) (mmol/L)** | 137.98 ± 4.29 (53) | 138.26 ± 7.50 (38) | 136.60 ± 12.98 (15) | 143.27 ± 8.60 (11) |
| **Potassium (K) (mmol/L)** | 4.45 ± 0.60 (53) | 4.88 ± 1.11 (38) | 4.71± 0.75 (15) | 5.55 ± 1.20 (11) |
| **Creatinine (Cr) (mg/dL)** | 1.11± 0.59 (54) | 0.66 ± 0.14 (38) | 1.06 ± 0.95 (15) | 0.65 ± 0.11 (11) |
| **Urea (mg/dL)** | 28.66 ± 23.10 (53) | 15.18 ± 3.40 (38) | 21.14 ± 17.82 (14) | 20.91 ± 13.40 (11) |

A statistically significant difference was detected between the methylated *KLOTHO* hypertensive patients and unmethylated *KLOTHO* control subjects for potassium (K) (p= 0,0014) (Figure 4).

### The Association Between *ARNTL* and *KLOTHO* Methylation Status

*ARNTL* and *KLOTHO* were both methylated in 53 out of 115 subjects (46.1%) and unmethylated in 10 out of the 115 subjects (8.8%) (Table 4). The association between methylation status of *KLOTHO* and *ARNTL* genes was not statistically significant (p > 0.05).

**Table 3.** Mean Values of Biochemical Parameters for *ARNTL* Gene

| Parameter | Methylated *ARNTL* | | Unmethylated *ARNTL* | |
|---|---|---|---|---|
| | Hypertensive subjects | Control Subjects | Hypertensive subjects | Control subjects |
| **Fasting glucose (mg/dL)** | 120.00 ± 70.48 (42) | 96.67 ± 15.54 (21) | 142.65 ± 87.50 (23) | 98.14 ± 6.02 (21) |
| **Triglyceride (mg/dL)** | 177.58 ±98.15 (33) | 132.50 ± 31.30 (20) | 172.06±91.38 (16) | 120.57±28.13 (21) |
| **Total cholesterol (mg/dL)** | 197.58 ± 59.89 (33) | 179.60 ±59.04 (20) | 195.07 ± 52.06 (14) | 168.14 ± 28.81 (21) |
| **HDL-C (mg/dL)** | 47.55 ± 15.91 (33) | 48.55 ± 8.75 (20) | 41.87 ± 10.33 (15) | 53.48 ± 12.15 (21) |
| **LDL-C (mg/dL)** | 128.30 ± 60.90 (33) | 134.65 ± 35.32 (20) | 136.93 ± 71.65 (15) | 128.24 ± 26.19 (21) |
| **Sodium (Na) (mmol/L)** | 137.38 ± 8.36 (42) | 138.67 ± 6.63 (21) | 138.00 ±4.57 (22) | 141.00 ± 9.11 (21) |
| **Potassium (K) (mmol/L)** | 4.45 ± 0.70 (42) | 5.02 ± 1.05 (21) | 4.59± 0.57 (22) | 5.11 ± 1.30 (21) |
| **Creatinine (Cr) (mg/dL)** | 1.00± 0.51 (42) | 0.64 ± 0.15 (21) | 1.07 ± 0.52 (23) | 0.68 ± 0.12 (21) |
| **Urea (mg/dL)** | 26.78 ± 23.29 (40) | 17.95 ± 10.51 (21) | 23.22 ± 13.49 (23) | 15.76 ± 2.95 (21) |

**Table 4.** The Interaction Between *ARNTL* and *KLOTHO* Methylation Status

| | Subjects (p + c) | | |
|---|---|---|---|
| **Methylation Status of *ARNTL*+ *KLOTHO* Genes** | **Count** | ***%*** | **p value** |
| ***ARNTL* Methylated+ *KLOTHO* Methylated** | 53 | 46.1% | p>0.05 |
| ***ARNTL* Methylated+ *KLOTHO* Unmethylated** | 14 | 12.1% | |
| ***ARNTL* Unmethylated+ *KLOTHO* Methylated** | 38 | 33.0% | |
| ***ARNTL* Unmethylated+ *KLOTHO* Unmethylated** | 10 | 8.8% | |
| **Total** | 115 | 100% | |

(% in total subjects containing patients (p) and controls (c))

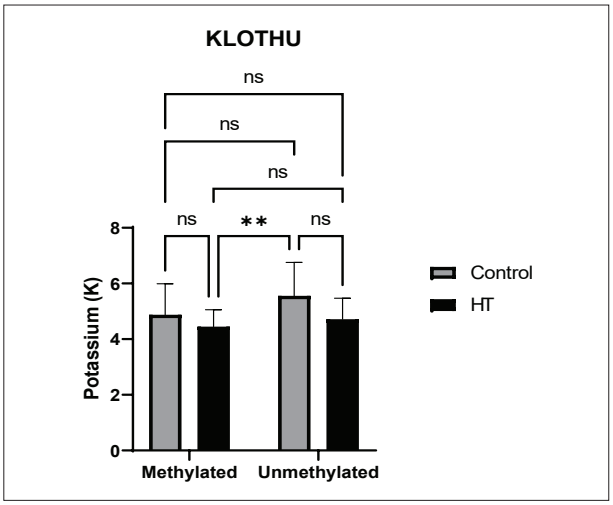


**Figure 4.** The association between the methylation status of *KLOTHO* and hypertension for potassium (K) (ns: not significant, **: p-value <0,05)

## DISCUSSION

Hypertension is an important risk factor for kidney disease and cardiovascular diseases such as stroke, heart failure (HF) and arrhythmias [1,2]. The risk of hypertension significantly increases with age in both men and women [25]. However, the risk of hypertension exhibits variation based on gender. Particularly, the incidence of hypertension was found to be higher in postmenopausal women than those age-matched men and premenopausal women. It was suggested that obesity-mediated sympathetic activity, low estrogen level, and high renin-angiotensin system (RAS) activity might contribute to postmenopausal hypertension [26].

Human KL protein exists as a full-length single-pass transmembrane protein and soluble KL The soluble KL protein can be found in blood, urine, and cerebrospinal fluid [8,9]. A large proportion of soluble KL is provided from the kidney [7]. KL suppresses oxidative stress and aldosterone secretion, inhibits insulin/IGF-1 (insulin-like growth factor 1) and Wnt (wingless-related integration site) pathways, apoptosis, fibrosis and cell senescence, and regulates calcium-phosphate homeostasis [1].

*KL*-deficient mice were indicated to display aging-related phenotypes, including hypertension, arterial stiffness, and chronic kidney disease (CKD) [27]. The silencing of *KL* gene was found to be related to hypertension and high level of aldosterone in human adrenocortical cells [28]. It was indicated that membranous and soluble KL can inhibit Wnt/β-mediated activation of RAS by blocking the binding of Wnt ligands, and thus preserving kidney function and normalizing BP. Therefore, it was suggested that KL has an ameliatory effect on hypertension in CKD patients [29]. The soluble KL levels were found to be positively correlated with high-density lipoprotein-cholesterol (HDL-

C) and negatively correlated with serum triglycerides in controls, and inversely correlated with body mass index (BMI) in hypertensive patients [3]. It was suggested that low plasma KL levels could increase total body sodium in patients, leading to chronic inflammation and high blood pressure. In addition, it was indicated that this process could be related to CKD patients with low serum KL levels [7].

It was reported that women with low serum KL concentration have a high risk of postmenopausal hypertension. It was suggested that serum KL concentration may be a significant biomarker to evaluate the risk of hypertension in postmenopausal women [26]. Several studies reported that the lower KL concentration was related to higher blood pressure, increasing the risk of hypertension [30,31]. In contrast, serum KL levels were detected not to be significantly different between subjects with and without hypertension in the general Chinese population [27]. Furthermore, a larger population-based study indicated no association between serum KL concentration and blood pressure [32,33]. In our study, we found no statistically significant link between *KL* methylation and hypertension (P>0.05). The difference between the studies' findings could be the result of a limited sample size or the age of the population. Since changes in *KL* expression have been reported during aging, focusing on large-scale studies that evaluate the association between KL concentration, *KL* methylation, and hypertension in age-matched patients with different disease conditions and control subjects would be important. The soluble KL protein is produced by the kidney. Therefore, it is claimed that, in cases where the kidneys are normally functional and soluble KL protein is at normal levels, *KL* expression may vary in other tissues. Thus, it is suggested that *KL* expression in other tissues may decline due to different factors, including genetic variants and epigenetic alteration [34]. Therefore, this information highlights how this situation should be taken into consideration in further studies.

Particularly, it is defended that the effect of genetic polymorphisms in the *KL* gene on blood pressure should not be ignored [27]. Individuals with the GA+AA genotype of *KL* G-395A polymorphism were found to have a lower SBP, relative to the individuals with the GG genotype [35]. Thus, it was suggested that the A variant of *KL* G-395A might prevent the development of essential hypertension by increasing the *KL* levels [5, 13]. The uncertain mechanism affecting the human *KL* promoter region was indicated to inhibit *KL* gene expression in HEK293 cells [36]. The *KL* promoter region has been reported to be sensitive to DNA methylation [37, 38]. The *KL-VS* variant contains six variants, and two variants thereof are located in exon 2 cause amino acid substitutions, F352V and C370S. The *KL-VS* variant has been associated with enhanced SBP, serum cholesterol and cardiovascular disease in Baltimore Caucasian and African American subjects [39-41]. A meta-analysis has indicated that the G-395A, C1818T SNPs of the *KL* gene might be associated with a hypertension risk [2]. The F352V (rs9536314 T> G) polymorphism of the *KL* gene was correlated with salt-sensitive hypertension in an Italian study [14]. The *KL* rs9536314 and rs564481 polymorphisms were associated with increasing levels of HDL-C in females [42]. High HDL-C levels were suggested to be protective against *KL* dysfunction [40]. Particularly, HDL and KL have been indicated to participate in the regulation of similar signaling pathways, including both molecules that promote nitric oxide (NO) synthesis, angiogenesis, and inhibit apoptosis, and insulin signaling in cell culture models [42, 43]. The fasting glucose was found to be predominantly higher in women with the T allele of *KL* rs564481 relative to non-carriers in Japanese [12] and Korean women [11]. It was reported that KL reduces the levels of serum creatinine. The decrease in *KL* expression has been related to aging-related kidney damage. Particularly, enhance in serum creatinine was indicated to be a diagnostic factor of acute kidney injury (AKI) [29]. Although several studies have reported a relationship between some biochemical parameters and KL protein, in our study, the relationship between the methylation status of *KL* and fasting glucose, triglyceride, total cholesterol, HDL-C, LDL-C, Na, K, Cr and Urea levels, were not significant ($p > 0.05$). It is considered that these contradictory results may be due to different genetic backgrounds in different populations. However, levels of K were significantly different between the methylated *KL* hypertensive patients and unmethylated *KL* control subjects (p= 0,0014).

The circadian clock regulates the circadian oscillation of human blood flow by enhancing it during the day and decreasing it during the night [44]. Circadian clock genes were implicated in modulating many processes involved in the regulation of the blood pressure in the kidney, heart, vasculature, and metabolic organs [17]. It has been reported that the circadian oscillation of blood pressure decreases with age [45].

The global *ARNTL* knockout (KO) mice were found to have lower BP with impaired BP rhythm. It was shown that decreased BP can be associated with changes in the vasculature in *ARNTL* KO mice [18]. Furthermore, the global *ARNTL* KO mice were indicated to lose diurnal sodium excretion [46]. Smooth muscle components of the blood vessel wall provide sufficient blood flow to organs and blood pressure homeostasis by regulating its contractile state. Smooth muscle-specific *ARNTL* KO male mice were shown to exhibit decreased BP and less impaired BP rhythm [16]. *ARNTL* rs3816358 polymorphism has been related to non-dipper hypertension in young hypertensive

patients [47]. In rats, the *ARNTL* gene was stated to be situated in hypertension susceptibility loci. The 18477-T/G variant in the *ARNTL* promotor was reported to significantly decrease the Gata-4-mediated transcriptional activation of the *ARNTL* promotor. Moreover, in respect of this polymorphism, *ARNTL* promoter activity was indicated to be consistently 2-fold higher in normotensive Wistar-Kyoto (WKY) rats than in spontaneously hypertensive rats (SHR) [21]. It is suggested that hypertensive patients with the GG genotype of *ARNTL* A1420G may exhibit high nighttime SBP [48]. The essential arterial hypertension (EAH) patients with GG genotype of the circadian locomotor output cycles protein kaput (*CLOCK)* 257TG polymorphism were found to exhibit lower *ARNTL* expression than EAH patients with other genotypes at 9.00, 13:00, and 17:00 time points [49]. The downregulation of *ARNTL* levels was detected in hypertensive female patients [4]. Therefore, all these findings support that *ARNTL* is an essential factor during the development of hypertension. However, the mechanisms underlying ''circadian genes'' in the regulation of blood pressure have not been comprehended yet. According to our study, the *ARNTL* gene was methylated in 68.7% of the hypertensive subjects and the relationship between methylation status and hypertension was not significant ($p > 0.05$). Furthermore, the relationship between the methylation status of *ARNTL* and fasting glucose, triglyceride, total cholesterol, HDL-C, LDL-C, Na, K, Cr and Urea levels, were found to not be significant ($p>0.05$).

In summary, we did not identify a statistically significant association between *KLOTHO* and *ARNTL* methylation status and hypertension and their association with fasting blood sugar, triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, Na, K, Cr, Urea levels in hypertensive patients. The limitations of our study are the age mismatch between patients and controls, and the small number of participants. Since several factors such as age, gender and obesity and diabetes are risk factors for hypertension, patients and controls matched by age, gender, and BMI should be included in future studies to minimize the impact of these factors on the results [3]. Therefore, as mentioned in this study, these conditions should not be ignored in future large population-based studies.

## CONCLUSION

Several studies have revealed the influence of *KL* and *ARNTL* polymorphisms and altered gene expression on hypertensive patients. Considering these results we focused in this study on understanding the importance of the methylation status of these genes in hypertension and their interaction with biochemical parameters. Although we could not detect a significant difference in *KL* and *ARNTL* methylation status in hypertension patients and controls, this study will shed light on the analysis and determination of new epigenetic pathways involving *KL* and *ARNTL* genes. Furthermore, except for *ARNTL*, considering that other core circadian clock genes may participate in the onset and progression of hypertension, their epigenetic analysis may provide further information on the determination of the mechanisms involved in the hypertension progress.

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