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Effect of circadian rhythms disturbances in clock gene 3111T/C

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Abstract:

Venous blood samples were collected from 50 people (40 people with insomnia and 10 healthy people) to detect the association between lipid profile, hormone melatonin and clock protein with circadian locomotor output cycles protein kaput (clock) 3111T/C gene polymorphism, 50 people were divided in to males and females, and then each group was divided in to three group:_ the first group had the TT genotype, The second group had the TC genotype and the third group had the CC genotype detected by polymerase chain reaction (PCR) the enzymatic method was used to measure the lipid profile and the ELISA technique was used to measure the hormone melatonin and the clock protein. The statistical analysis was carried out using the SPSS program. The results indicated that cholesterol, triglyceride and LDL were elevated in the TC and CC genotypes compared to their decrease in the TT genotype. Whereas melatonin and clock protein were decreased in the TC and CC genotypes compared to its high in the TT genotype.

Keywords: clock gene 3111T/C, clock protein, hormone Melatonin, cholesterol, triglyceride, circadian clock

Introduction:

The biological clock is the natural timings of living organisms that are made up of specific molecules and proteins that interacts with cells throughout the body (Lyons *et al.*, 2019) The name biological clock is derived from the Latin circa which means around or circle, and Dian, which means day (Serin and Tek 2019) circadian rhythms are regulated by the molecular clock, a family of proteins that work together in transcriptional feedback loop. Several studies have indicated that there is a relationship between the clock 3111T/c single

_Nucleotide polymorphism and circadian disorders located in the untranslated region (3_UTR) Agrion that has been shown to be very important for mRNA stability. Alterations in this region can lead to increased stability or perturbation of genetic products (Ozbum *et al.*, 2016) where Semenova *et al.,* (2017) indicated that clock gene 3111T/c is affected in people who suffer from insomnia sleep disturbances and circadian irregularities and they also discovered the fact that the C allele is associated with insomnia, also Bandin *et al.,* (2013) noted the influence of circadian rhythms in gene disorders clock 3111T/c when he compared the normal genotype TT who showed more robust and regular patterns of circadian rhythms while patients who carry the c allele showed significant disturbances in the circadian clock disturbances and less stability, according to the CFL assessment, as they were less active in the morning and more sleepy during the day alteration Semenova *et al.*, (2018) of the hormone melatonin, which is a key component of the circadian clock mechanism, was observed between the association of the circadian rhythm of melatonin with the 3111T/C gene polymorphism around the clock in Caucasian women with insomnia, the TT genotype of the insomniac women compared to the control group had a higher melatonin level in the morning and lower melatonin at night, the TT genotype of A Sian women with insomnia also observed lower levels of melatonin compared to the day, evening and night control and Tsuzaki *et al.,* (2010) indicated that the 3111T/c clock gene is associated with disorders of lipid regulation cholesterol, glucose and metabolism and leads to the presence of small, low density lipoprotein (sdLDL) in the circulation, a defect in lipid metabolism, also Semenova *et al.,* (2021) found the clock gene 3111T/C associated with increased cholesterol and low-density lipoprotein levels (LDL)

Materials and Methods

This study included 50 volunteers, 40 with insomnia and 10 control, between the ages of 22_91 years. Laboratory tests (lipid profile, clock protein, melatonin hormone, genetic test) and statistics were performed

Blood samples

A Between 8_11 am and after 12 hours of overnight fasting venous blood samples (the cubital vein) were taken into two tubes: The first tube contained tripotassium ethylene Día monetaristic acid was used for genetic testing, The second tube contained Gel and clot Activator and it was centrifuged then used serum to measure total cholesterol, TG, HDL and LDL(Mohammed *et al*., 2017; Semenova *et al.,* 2017) B_Between 9 and 11 pm, samples of

venous blood (the cubital vein) were taken to a tube containing Gel and clot Activator for clock protein and melatonin hormone testing (Rzepka_Migut *et al.*, 2020) .

Molecular and genetic examination

DNA was extracted from the blood of all samples included in the study, which are 50 samples using the modified method (Lranpur and Esmailizadeh, 2010). Then it was amplified clock gene 3111T/c by adding 100 ng of template DNA and 10 picomoles of special primers for the clock gene 3111T/C supplied by Macrogen were added to the master mix contents, this primer was prepared as it comes, forward (5-TCC AGC AGT TTC ATG AGA TGC -3) and reverse (5- GAG GTC ATT TCA TAG CTG AGC-3) the reaction tubes were inserted in to the thermal cycler device to conduct the multiplication reaction using the special program for the reaction as (Semenova *et al.,* 2021) shown in table (1)

Table (1) phases of the PCR polymerase chain Reaction program

No.	Stage	Temperature	Time	Cyclenumber
1	Initial denaturation	95	5 min	1
2	Denaturation	95	45 sec	35
3	Annealing	56	1 min	35
4	Extension primers	72	1 min	35
5	Final extension	95	45 sec	1
		65	1 min	
		72	7 min	

After the reaction was completed, the tubes were removed from the thermocycler device with, PCR product was 221 bp in size, 5 μl were with drawn and incubated with the restriction enzyme (Bsp 1286I) with a concentrated of 5 units supplied by Biolaps company for 3 hours at 37°C, C ellale was cut by Bsp 1286I. The sample is then loaded in to a well of a agarose gel with a DNA ladder supplied by Biolabs, then the samples are migrated by running the electrophoresis device for 90_75 minutes, adding 1µl of red safe day, and finally imaging the gel with a Gel documentation device (Altaee 2018)

ELISA technique to measure the hormone melatonin and clock protein

A ready _made kit was used to measure the clock protein from the manufacturer sun long Biotech with the number SL3124Hu and the ready _made kit of measure me from the manufacturer sun long Biotech that carries the number SL1169Hu and using the device Elisa. The blood was drawn and placed in special tubes and left at room temperature to form a clot

for 15 minute then the tubes were placed in a centrifuge for 20 minutes and 3000 rpm after which the serum was separated and placed in a cup serum at -20° prepared a standard according to the kit method for each measurement and put in the first 5 of the well for the Microelisa, leaving the 6 well as blanks control, 40 µl of buffer solution to dilute and 10 µl of blood sample in to the well. lt was left to incubate for 30 minutes at 37

then the washing process was carried out 5 times then 50 µl of the reagent HRR _conjugate was added to each well except for the control well and left to incubate for 30 minutes at 37C and the washing process was 5 times after incubation 50 µl of solution chromogen solution A and 50 ml of solution B were added to each well, mixed with gentle stirring and incubated at 37C for 15 min. finally 50µL from stop solution were added to each molecule to finish the reaction. we note the Colour change from blue to yellow and then read the absorbance O.D at 450 nm using a plate reader Microtiter plate Reader (Alhajj and Farhana 2022; Alhamadani *et al.,* 2017)

Biochemical analyzes Measure lipid profile

Measuring were total cholesterol, LDL,HDL and triglycerides in one auto work method (Auto chemistry Analyzer) and with one special device called DONGJIU manufactured by the company(China/ Ray to) and according to the manufacturer ś instructions for several work related to the device and the method of work was followed up as follows:

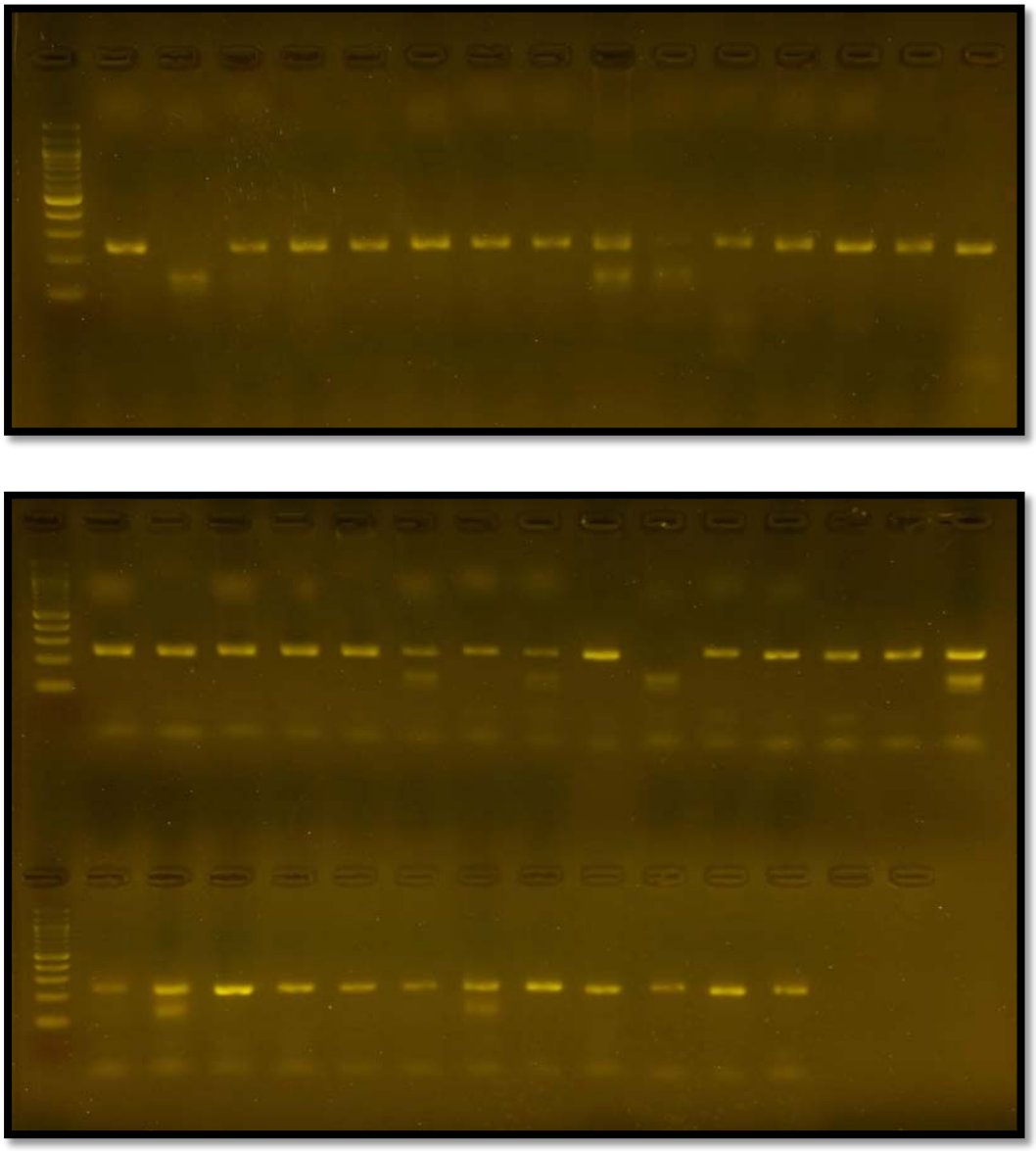
The blood was drawn after a 12-hour fast and the blood was placed in special tubes and left at room temperature for 15 minutes to form a clot then the blood was placed the tubes were placed in a centrifuge for 5 minutes at 3000rpm the serum was separated and a about 1000 microliters were placed in small tubes special to the device called Hitachi cup. These tube were placed in the designated place in the (DONGJIU) device and the device was controlled to perform the test

Statement Analysis

Using the program statistical package for the social sciences(Spss) version No. 25 to find the mean, standard deviation and error deviation of the mean and to find the relationship between the variables the date was processed with the Pearson two tail program at a significat value $p<0.01$ and $p<0.05$

Results and Discussion

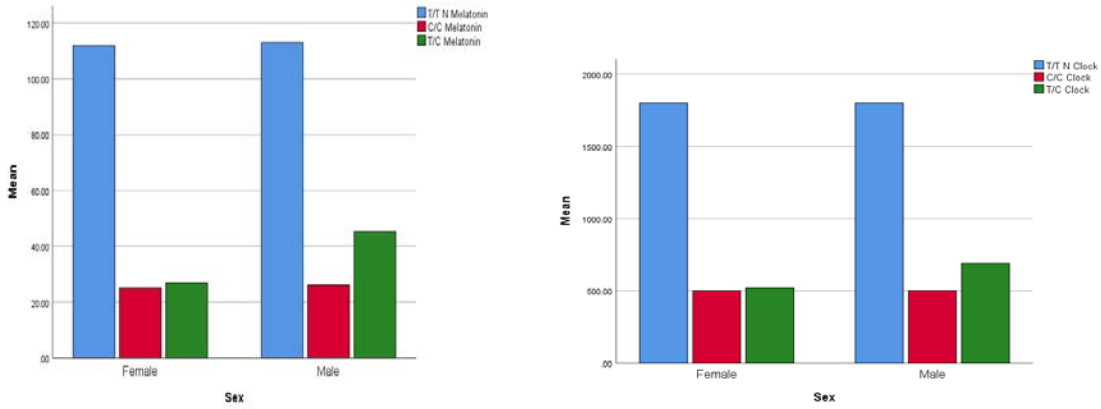
The results of applying the PCR technique showed three types of genotype for the clock gene 3111T/C: Normal Homozygous TT, Heterozygous TC, Mutant Homozygous CC as shown in the figure(1)



Figure(1)The result of the PCR reaction with a size of 221pb with the trimer enzyme (Bsp12861)

The results as shown in figure (2) when measuring the hormone melatonin, indicate a clear decrease in the CC, TC genotype compared to the normal TT, Melatonin is a sleep-inducing hormone secreted by the pineal gland at night, whose primary function is to provide a time signal to regulate circadian rhythms (Erland and Saxena 2017) where the concentration of the hormone melatonin is insomnia patients with genotype CC 2.5-26pg/ml while its concentration in normal people was more than 100pg/ml, this is due to the fact that circadian rhythm disturbance, insomnia and exposure to light at night decrease melatonin production, Which causes sleep disturbances and changes the circadian timing system (shafi and Knudsen 2019) this matches what semenova etal 2018) found when measuring the concentration of the hormone melatonin in people with insomnia, where he noticed a higher concentration in the morning and a lower level of concentration at night compared to normal people with the genotype TT, It is also similar to what Hajak *et al.*,(1995) found, where his results showed

that the circadian rhythm of melatonin secretion is disturbed in patients with primary and chronic insomnia and that the secretion of melatonin in the octanal plasma is increasing affected the longer the patients in ability to maintain a regular sleep pattern. It also found a relationship between the circadian clock disorders of people with insomnia with the clock protein where figure(2) indicates a decrease in the clock protein in infected and pregnant persons compared to normal persons. Where BMALL has been identified as the asymmetric partner of clock as they together from the CLOCK:BMALL transcription activator complex, they are both positive elements of transcription mechanisms through self-regulation feedback loops, where the concentration in the genotype TC 600.5-574 pg/ml for females and males compared to normal people which reached 1800 pg /m/, this is similar to the finding of Mongrain *et al*., (2011) where he noted that circadian rhythm disturbances, sleep loss and insomnia reduce the link between circadian rhythms and circadian rhythms CLOCK:BMALL, sleep disturbances and insomnia also differ coding in the CRY1 gene core in the circadian clock gene that represses the circadian clock- activating protein clock: Bmal1 transcriptionally there by reducing expression of key transcription targets and prolonging the duration of circadian molecular rhythms (Patke *et al.,* 2017) this decrease in clock protein may also be due to circadian disturbances that shift and alter the regulation of clock: Bmal1and cry over the light-dark cycles and which disrupt the kinases, Rev-erba and RoRa genes by inducing changes in per (Khan *et al*., 2018)

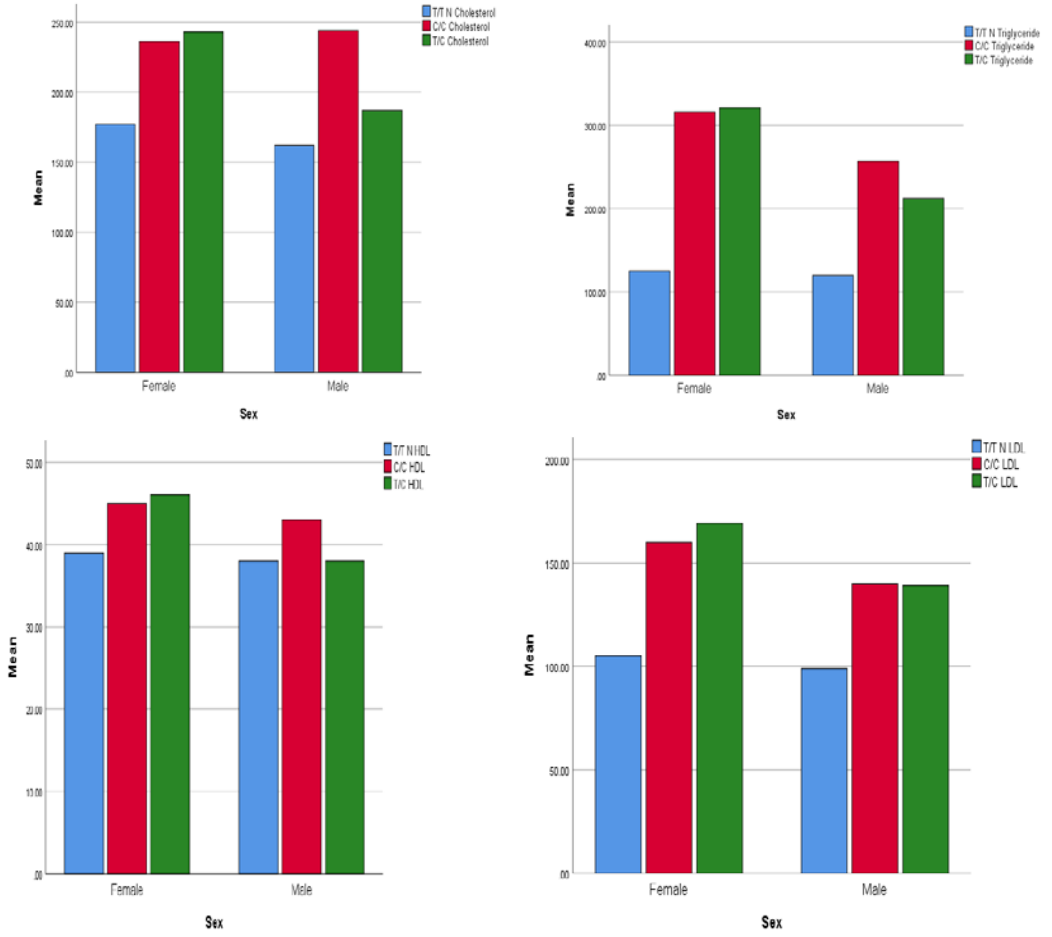


Figure(2) Melatinin and clock measurement of the TT, CC, TC genotype

Figure(3) when measuring cholesterol indicates an increase in the CC, TC genotype compared to the normal TT genotype, where disturbed sleep is closely associated with an increased risk of metabolic diseases complete lipid profile abnormalities and increased blood cholesterol levels.(Mattos *et al.,* 2020, Xing *et al*., 2020) where the concentration of cholesterol in the CC genotype was 244,236 mg/dL for females and males, while the normal

pattern was 163, 152mg/dL females and males, and this is due to the fact that lack of sleep shifts the balance of hormones from those that promote feelings of fullness to others that cause feeling of hunger, as it increases receptors that promote appetite (cooper *et al*., 2018) the body produces a lot of cortisol and appetite-boosting hormone which promotes appetite, and in contrast, very little of the hormone lepton, which regulates body weight which leads to hormonal imbalance and cholesterol imbalance. (Hirot su *et al*., 2015) figure(3) also indicates a high concentration of triglycerides in the TC, CC genotype compared to the TT genotype, which may be due to the fact that staying up late at night affect the intestinal microflora that regulates body composition and this leads to an increase in the wake of the dietary fatty acids and fat storage and thus causes obesity (Li *et al*., 2019) where the concentration of triglycerides in the TC genotype for females reached 310mg/dL, compared to the TT genotype for famales which reached 113 mg/dl where a study confirms that insomnia and staying up until the morning hours increase appetite for foods rich in triglycerides (Martchenko and Brubaker 2021) this is consistent with what cedrenes (2015) found when studying 15 healthy young men where a single night of lack of sleep to changes favoring fat storage. It was also discovered that skeletal muscle protein decreased and lipid-promoting protein increased in response to sleep loss (Katagiri *et al*., 2014) figure(3) indicates a higher Low- Density lipoprotein in the TC, CC genotype compared to TT

LDL is a law-density lipoprotein called bad cholesterol due to the fact that it sticks to the arterial well when high blood levels are present and thus leads to the risk of heart attacks and strokes, It's percentage in the male CC genotype 140mg/dL compared to the male TT genotype which reached 100mg/dL this rise may be due to staying up all night or sleeping less than 6 hours a day, which increases LDL(Mosca and Aggarwal 2012) the table indicate that the reasults are some what convergence for high density lipoprotein as we note that the results are identical in the TC, TT genotype which is good cholesterol.These disorders in the lipit profile may be due to circadian disorders, insomnia and lack of sleep and not due to eating high-fat food and lack of exercise (Joo etal 2019)



Figure(3)cholesterol,LDL,HDL and triglycerides measurement of the TT,CC,TC genotype

Table(2)the results of the statistical and lysis to find the error and standard deviation

Descriptive Statistics			
	Mean		Std. Deviation
	Statistic	Std. Error	Statistic
T/T N Sex	1.4545	.15746	.52223
T/T N Age	49.5000	5.59414	17.69024
T/T N Weight	76.8000	3.87528	12.25470
T/T N Cholesterol	157.0000	2.78089	8.79394
T/T N Triglyceride	109.0000	3.40588	10.77033
T/T N HDL	36.7000	.55877	1.76698
T/T N LDL	93.2000	2.26961	7.17712
T/T N VLDL	21.8000	.68118	2.15407
T/T N Risk Factor	4.2730	.06643	.21008
T/T N Melatonin	117.5500	1.54641	4.89018
T/T N Clock	1832.9000	14.39788	45.53009
C/C Sex	1.5000	.50000	.70711
C/C Age	57.0000	7.00000	9.89949
C/C Weight	113.0000	8.00000	11.31371
C/C Cholesterol	240.0000	4.00000	5.65685
C/C Triglyceride	286.5000	29.50000	41.71930

C/C HDL	44.0000	1.00000	1.41421
C/C LDL	150.0000	10.00000	14.14214
C/C VLDL	57.3000	5.90000	8.34386
C/C Risk Factor	5.4550	.21500	.30406
C/C Melatonin	25.5250	.52500	.74246
C/C Clock	500.5000	.50000	.70711
T/C Sex	1.4286	.20203	.53452
T/C Age	47.8571	5.16595	13.66783
T/C Weight	110.1429	4.88159	12.91548
T/C Cholesterol	223.0000	9.56432	25.30481
T/C Triglyceride	244.4286	25.54348	67.58170
T/C HDL	42.8571	1.40456	3.71612
T/C LDL	141.8571	7.46876	19.76047
T/C VLDL	48.8857	5.10870	13.51634
T/C Risk Factor	5.1943	.11660	.30848
T/C Melatonin	34.7686	2.98709	7.90310
T/C Clock	589.1429	23.93586	63.32832
Valid N (listwise)			

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