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Association of C-allele carrier Genotype of *SLCO1B1* gene 521T>C Polymorphism and Statins Related Myopathy in a Sample of Iraqi Patients

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Abstract: The gene *SLCO1B1* 521 T>C is a crucial gene single nucleotide polymorphism (SNP) concerning of a several medications transport enzyme OATP1B1 action variation such as statins drugs that may cause a serious side effect (myopathy), Iraq lack from recent studies about this variation related to statins side effect myopathy.The study of the *SLCO1B1* gene 521 T>C polymorphism effect on Iraqis patients taken statins differ in showing myopathy as a side effect of statin. Settings and Design considered An only treated case-control study. The study of the effect of T521C of *SLCO1B1* gene polymorphism and myopathy carried by collect venous human blood (3) ml of (96) samples divided to (48) as control and (48) as case. DNA extraction carried by (wizibiosolutions Korean kit for DNA extraction) and detection by Gel-Electrophoresis, nanodrop device used to adjust purity and concentration proper to next step, TaqMan Real-Time Polymerase Chain Reaction (TaqMan RT-PCR) carried by Real-Time PCR System . Myopathy determined by a history of the patient, clinical examination. Highly significant difference p-value (0.0001<0.01) in genotype carry C-allele between control and case. Genotype (CC) was zero in control compared with cases, all (CC) genotype 5 patients with myopathy was (100%) in case. Genotype (TC) only 5 patients represent (15.6%) was in control parallel to 27 patients represent (84.4%) in case. Genotype (TT) wild type was 43 patients (72.9%) in control compare to only 16 case patients represent (27.1%). All numbers refer to a strong correlation between genotypes carry C-allele (100%, 84.4%) and case (patients with myopathy) against control only (15.6%) of (TC) genotype without myopathy. The significant risk of myopathy of (CC) genotype compare with (TT) genotype when (n=96) odd ratio (17.2) at (95%) Cl between (5.7051 to 51.8551) and P (0.0001) indicate the risk of development of myopathy for C-allele carrier genotype. C-allele considered a risk factor for patients taken statin lead to develop of myopathy in Iraq.

Keywords: *SLCO1B1*, 521T>C, Polymorphism, SNP.

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Introduction:

Statins firstly produced at 1982 are lipid-lowering medicaments mostly used in the clinic (first line of treatment hyperlipidaemia) to improve cardiovascular diseases (1). The main side effect of statins is myopathy found in 5-25 percent of cases taken statins (2).

SLCO1B1 gene is a solute carrier organic (SLCO) anion transporter family member 1B1 (*SLCO1B1*) gene encodes for a membrane-bound sodium-independent organic anion transporter protein (OATP1B1) mediates active intracellular hepatic transport of anionic compounds (2).

The common T521C variant rs4149056 of *SLCO1B1* gene produces a (Valine174Alanine) substitution. This

substitution leads to decrease OATP1B1 activity by the minor (C) allele at this locus has been associated with decreased transport function lead to myopathy (3). C-allele frequency is about (5-20 %) in most populations (4).

Testing for known genetic variants that affect drug metabolism can potentially enhance therapeutic response to medication, reduce side effects, and optimize treatment of disease, while this can positively affect both disease-specific outcomes and patient satisfaction (5,6). The study of the effect of T521C of *SLCO1B1* gene polymorphism and myopathy in Iraq patients taken statin to avoid this harmful side effect.

Materials and Methods:

Study groups of ninety-six samples were randomly collected from Iraqi patients take statin medicaments of different medical institutes in three large cities: (Imam Hussein Medical City in Karbala City, Baghdad Medical City in Baghdad City, AL-Kawther Primary Medical Health Centre in Babylon City). The period was from the first of November 2017 to April 2018.

Ethical issues depended on approval of a scientific committee of the Institute of Genetic Engineering and Biotechnology (University of Baghdad, Iraq), The approval of Ministry of Health and Environment, Iraq and the method and objectives of this research were showed to all participating in the study to gain their verbal acceptance.

The sample divided to (48) patients show myopathy as side effect of statin treatment check by clinical findings and (48) patients that taking a statin with no myopathy as a side effect for comparison.

Inclusion Criteria: Groups age less than 60 years old because older patients start to show ageing causes myopathy mismatch with a statin-related myopathy side effect, patients take any type of statins at least 3 months (required period to gate statin-related side effect)

Exclusion Criteria: states of not statin-related causes of myopathy. On the other hand, drug-drug interaction avoided in patients taking the following groups of Drugs: Cyclosporine, Macrolides, Warfarin, amiodarone, Fibrates, Rifampicin, Verapamil, Azoles, Angiotensin Converting Enzyme Inhibitors and Anti protease with or without muscle pain.

DNA Extraction:

DNA extracted by using DNA Extraction Kit. Wizbiosolutions from (2.5 ml) EDTA anticoagulant tube Peripheral venous blood by using instruction procedure for frozen blood for (96) samples.

DNA Estimation and Determining:

The presenting, concentration and purity must adjust for all DNA samples before the PCR step. The presence of DNA determined by Agarose Gel Electrophoresis procedure (7). Adjusting of DNA Concentration and Purity by measure use micro-measure devise Q3000 UV Spectrophotometer were read with nanodrop at wavelength 260 nm. The concentration of the DNA in ng/ul of the sample must adjust concentration between 3-20 ng/ul by use equation Concentration1* Volume1= Concentration2 * Volume2.

The determining the purity of DNA, readings were taken at

wavelength 280 nm. The purities of DNA would be Pure DNA: Absorbance at 260/Absorbance at 280 = 1.7 --- 1.9. All samples accurate frozen at -20C to next step.

SNP Identification:

The determination of *SLCO1B1* 521 T>C SNP type according to TaqMan Drug Metabolism Genotyping Assay no. C_30633906_10 Kit. Used Real-Time PCR System. Reaction carried out by wet DNA delivery method, total volume of reaction mixture for each well of (PCR) plate 25 µl contain: recently prepare for each run by mix 1.25µl of 20X Taqman Drug Metabolism Genotyping Assay must be vortex and centrifuge before use plus 12.5µl of 2X TaqMan Universal PCR Master Mix gently swirl before use, both mix in new tube standby, then flick, invert and centrifuge before adding 13.75µl from it into each well contain DNA amount with a range of 3-20 ng per well dilute with DNase-free water give volume 11.25µl. Each run must contain at least 2 no template controls to ensure optimal analysis and troubleshooting abilities.

When all wells reaction mixture filled, Plate undergoes slow vortex and enter Thermal Cycler that recently programmed its software to experiment reaction steps include: holding enzyme activation at 95 ^{0}C for 10 minutes, 50 cycle (Denaturing 15 second at 92 ^{0}C, Anneal 90 second at 60 ^{0}C).

The analysis of TaqMan Polymerase Chain Reaction Products according to sequence detection system (SDS) version 2.0.1. Software.

Both stains curves rise indicate heterozygous (T,C) alleles present as show in (Figure 1).

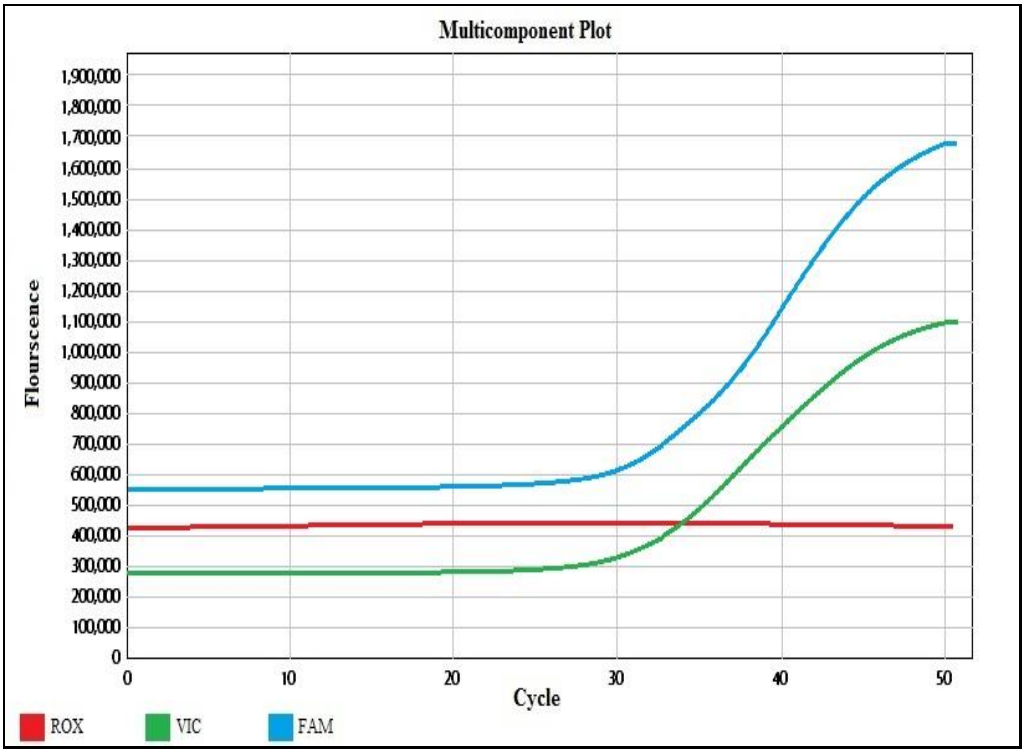

Figure (1) : Detection of TaqMan RT-PCR product showed two curves rising for both FAM stain (blue) and VIC stain (green) at cycle number 30 indicate Heterozygous in *SLCO1B1* 521T >C SNP give (TC) genotype of two different alleles found (T, C).

Statistical analysis:

Used carried by SPSS version 21., chi square by fisher exact test used to compare between control and case. A p-value if ≤ 0.05 was considered as significant and p-value if ≤ 0.01 was considered as highly significant. Odds ratio >1 indicate risk develop in the future.

Results and Discussion:

Results of C-allele carry genotype differences between control and case showed in (Table 1).

Table (1): C-allele carry genotype differences between Patients on statin (without Myopathy) as control and Patients on statin (with statin related Myopathy) as case.

Genotype	Control (patients without statin related nyopathy)	Case (patients with statin related myopathy)	Total	Chi Square	P-Value	ORs(95% CI)
TT	43(72.9%)	16(27.1%)	59(100%)	12.52 **	0.0001**	1.573 (0.82-1.64)
TC	5(15.6%)	27(84.4%)	32(100%)	13.47 **	0.0001**	1.704 (0.92-1.64)
CC	0(0%)	5(100%)	5(100%)	15.00 **	0.0001**	2.00 (0.85-1.58)
Total	48 (50%)	48(50%)	96(100%)	---	---	---

The significant risk of myopathy of (CC) genotype compare with (TT) genotype when (n=96) odd ratio (2) at (95%) Cl between (0.85-1.58) and (P<0.01) indicate high risk of development of myopathy for C-allele homologous carrier genotype.

Results showed that there were highly significant difference (P value <0.01) in genotype (TT, TC and CC) between control and case. Genotype (CC) was zero in control compared with cases, all (CC) genotype (5 patients) with myopathy was (100%) in case. Genotype (TC) only (5 patients) represent (15.6%) was in control parallel to (27 patients) represent (84.4%) in case.

Genotype (TT) wild type was (43) patients (72.9%) in control compare only (16) case patients represent (27.1%). All numbers refer to a significant correlation between Genotypes carry C-allele (CC, TC) of case compared with control (TC) genotype without myopathy.

C-allele is associated with increased bioavailability of several statins drugs. The c.521T>C SNP is associated with an increase in plasma exposure to some OATP1B1 substrates, including several types of statins where the plasma exposures are substantially larger in individuals with the homozygous c.521CC genotype, whereas the exposures in c.521TC heterozygotes are usually increased only in moderate way (8,4). T521C (Val174Ala, rs4149056) in *SLCO1B1* interferes with localization of the transporter of the statin to the plasma membrane of hepatocytes and decrease its activity by decrease hydrophobic interaction at the core ,structural stability, then decrease the activity and affinity of protein to its substrates statins (9, 10).

The clinical significance of *SLCO1B1* c.521T>C is better exemplified by its effects on statins. Active simvastatic acid was about three fold greater in healthy individuals with

the 521TC genotype than in those who have TT genotype (2). Important in drug hepatic clearance and in general drug disposition, which is located on the basolateral (sinusoidal) membrane of hepatocytes. OATP1B1 is an important determining factor for the uptake of several HMG-CoA reductase (statins) inhibitors in the portal vein into hepatocytes (11,12).

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