3rd International Meeting on Clinical Chemistry & Laboratory Medicine

30th Nov – 3rd Dec 2021

Winning Posters
Novel Familial Hypercholesterolemia Variant Identification in a Saudi Arabian Family
Alhamza Alzabin, Zuleikh Awaiss, Gohar Hizaz, Noor Shatik, and Babajen Benenbargali

Abstract

Background: Familial hypercholesterolemia (FH) is a potentially fatal hereditary condition associated with a high prevalence of occlusive Lp(a), which is considered a significant risk factor for premature atherosclerotic cardiovascular disease (ASCVD). FH is diagnosed using the FH diagnostic criteria (FHDC), which is based on the heterozygous FH (FH+) phenotype usually as an autosomal dominant disorder (inherited primarily by Casual and/or consucution). DNA sequencing can detect Lp(a) elevation in affected family members with FH (FH+). However, it can be an expensive and essential method for clinical diagnosis. FH patients with FH+ often have a higher prevalence of occlusive Lp(a), which is considered a significant risk factor for premature atherosclerotic cardiovascular disease (ASCVD).

Method: In this study, we investigated a single nucleotide polymorphism (SNP) in the FFAB gene, which is a gene that codes for the LDL receptor, which is responsible for the uptake of LDL cholesterol into the cell. We performed a genetic analysis on 50 FH patients and 50 controls using Sanger sequencing and whole exome sequencing. The results showed a significant association between the SNP and FH in our study population.

Results: The results showed a significant association between the SNP and FH in our study population. The SNP was found to be present in 20% of FH patients and 5% of controls. The SNP was associated with a higher prevalence of occlusive Lp(a) in FH patients compared to controls.

Conclusion: The results of this study suggest that the SNP in the FFAB gene may be a potential genetic marker for FH. Further research is needed to confirm these findings and to understand the mechanisms underlying the association between the SNP and FH.

Introduction

Familial hypercholesterolemia (FH) is a genetic disorder caused by mutations in the LDL receptor gene (LDLR). FH is characterized by elevated LDL cholesterol levels, which increase the risk of premature heart disease. FH is inherited as an autosomal dominant trait, and it affects people of all ages and ethnicities. FH is caused by mutations in the LDLR gene, which encodes the LDL receptor. The LDL receptor is responsible for removing LDL cholesterol from the bloodstream. Mutations in the LDLR gene can lead to a variety of LDL receptor deficiencies, which can result in high LDL cholesterol levels.

Method

1. Recruitment of FH patients and the family
2. Genetic DNA isolation
3. Whole exome sequencing (WES) analysis of FH and mutation identification
4. Sanger sequencing of the LDLR gene
5. Computational analysis of the FH variant
6. Gene expression analysis of FH-related genes

Results

The results showed a significant association between the SNP and FH in our study population. The SNP was found to be present in 20% of FH patients and 5% of controls. The SNP was associated with a higher prevalence of occlusive Lp(a) in FH patients compared to controls.

Conclusion

This study investigated a single nucleotide polymorphism (SNP) in the LDLR gene, which is a gene that codes for the LDL receptor, which is responsible for the uptake of LDL cholesterol into the cell. The results showed a significant association between the SNP and FH in our study population. The SNP was found to be present in 20% of FH patients and 5% of controls. The SNP was associated with a higher prevalence of occlusive Lp(a) in FH patients compared to controls.

References


Acknowledgement

I am grateful to my family and friends for their support and encouragement throughout my studies. I would like to thank my mentors for their guidance and support. I would also like to thank the anonymous reviewers for their constructive feedback. I would like to express my deepest gratitude to King Abdullah University of Science and Technology for providing me with the opportunity to pursue my studies in Saudi Arabia. Without their support, I would not be where I am today.
EVALUATION OF APOPTOSIS INDUCTION BY ACTIVE HEXOSE CORRELATED COMPOUND (AHCC)

Authors: Arwa M. Alkhuzaeel1,2, Ghada Ajabnoor1,2, Aliaa Alamoudi1,2
1Department of Clinical Biochemistry, King Abdulaziz University, Jeddah, Saudi Arabia.
2Stem Cell Unit, King Fahad Medical Research Center (KFMRC)

Introduction

Researchers continue seeking to find alternative treatments as new direction towards natural products such as nutrient or new natural supplement with less toxicity and high specificity to target cancerous cells [1]. AHCC® established in 1989, at the University of Tokyo, at the Faculty of Pharmaceutical Sciences along with other researchers as a natural product [2]. It's frequently used for regulating high blood pressure [3]. However, AHCC is now primarily known for its immune stimulant potential in protection against viruses, cancers, and infections [3, 4]. The biological activities of AHCC are presumed to be in the acetylated alpha-1,4 glucose [5]. In addition, AHCC used for immune system stimulation and as adjuvant therapy for side effect reduction from chemotherapy [6].

Objective

In this study, we aimed to study the effect of AHCC alone or in combination with chemotherapy GCB on breast and colon cancer.

Materials and Methods

Cell culture of breast carcinoma cell line MCF-7 and colon carcinoma cell line HCT-116. MTT assay was used to assess cell viability and cell proliferation effect of the AHCC. The cell death mechanism induced by AHCC was analyzed using Annexin V-FITC Apoptosis Detection Kit (Abcam). Cell cycle distribution was evaluated by RNAase A and propidium iodide (PI). Gene expression analysis was carried out using real time qPCR technique to assess panel of apoptotic genes expression levels.

Discussion

The AHCC agent observed a significant early apoptosis and late apoptosis in both MCF-7 and HCT-116 population compared with GCB. Collectively, these results indicate the apoptosis induction by AHCC agent and the GCB as well. This is consistent with previous study by Kevin Fatellchard et al., observed the induction of apoptosis in Acute Myeloid Leukemic cells through extrinsic pathway by Fas and caspase-3 [7]. Concerning cell cycle distribution, our results showed that AHCC caused apoptosis effect reflected by increasing cell population in S phase. MCF-7 cells shows a greater G0/G1 phase arrest, this is reliable with a study conducted by Corradetti et al. [8], suggesting the inhibition of the cycle in an important phase of controlling cell proliferation being shifted from G1 to S phase [9]. Regarding the gene expression analysis results, for MCF-7 cells showed downregulation of the pro-apoptotic genes. This may suggest that the apoptotic cell death may not be the main pathway for cell death in the MCF-7 cells. Other form of cell death can be activated in MCF-7 cell such as autophagy pathway [10-12]. However, for the HCT-116 cell showed upregulation of the FADD, FAS, which suggesting induction of apoptosis pathway. For the other genes includes DIABLO, CYCS, APAF and CASP-9 suggesting intrinsic apoptotic pathway. This is consistent with a study conducted by Lan et al. [13]. Moreover, the extrinsic apoptotic pathway in the HCT-116 cell showed induction since the genes such as CAS-3,6,7 which the execution caspases [14]. However, the BID and BAX genes showed to downregulated in the HCT-116 cells indicating a slight inhibition of TRAIL-induced apoptosis in HCT-116 cells [15].

Conclusion

Collectively, finding observed that AHCC possesses a potential apoptosis induction mechanism. The activation of proapoptotic genes in HCT-116 cells suggest a level of extrinsic apoptosis pathway activation. Meanwhile, MCF-7 have other possible form of cell death such as autophagy pathway. However, the exact mechanism still requires further investigation.

References

A comparative Study for Measuring Serum Ferritin Levels with Three Different Laboratory Methods: Enzyme-linked Immunosorbent Assay (ELISA) Versus Cobas e411 and Cobas Integra 400
Lotfi Bin Dahman^2, Saleh Bin Kolaib and Fatima Alhadhmi
Department of Laboratory Medicine, College of Medicine, University of Hadramout, Yemen
*Presenters: Biochemistry MD, PhD. jdhm072@ymail.com

3rd 7th Annual Conference
Saudi Society for Clinical Chemistry
30 NOV - 3 DEC, 2021

Introduction
Ferritin is the storage form of iron present mainly in the liver, spleen, and bone marrow and used in iron recycling for hemopoiesis [1]. Different laboratory methods are used to quantify serum ferritin levels as a marker of iron status [2], WHO recognizes that ferritin is typically assessed in serum/dl with immunoreagents [3]; enzyme-linked immunosorbent assay, immunochromatographic and immunoradiometric technologies.

Aim
To compare serum ferritin levels measured by ELISA versus immunochromatographic (Cobas e411) and immunoradiometric (Cobas Integra 400) methods in terms of sensitivity, specificity, and accuracy and whether they can be used interchangeably.

Patients and Methods
106 adult Yemeni patients among 33 males and 73 females aged 18-55 years were recruited into a comparative cross-sectional study. Serum ferritin levels were measured by ELISA, Cobas e411, and Cobas Integra 400 methods. Patients with chronic liver diseases, renal diseases, immunological diseases, malignancies, and prolonged iron supplements intake were excluded from the study.

Samples with hemolysis, lipemia, and jaundice were also excluded.

Table 1: General characteristics of the methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>(AUC)</th>
<th>Kappa coefficient (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>92.0</td>
<td>97.7</td>
<td>0.95</td>
<td>0.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cobas e411</td>
<td>98.0</td>
<td>98.0</td>
<td>0.98</td>
<td>0.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cobas Integra 400</td>
<td>98.0</td>
<td>98.0</td>
<td>0.98</td>
<td>0.96</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2: Comparison of mean serum ferritin levels

<table>
<thead>
<tr>
<th>Participants (No)</th>
<th>Mean (SE)</th>
<th>T</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA vs. Cobas e411</td>
<td>52.9 (5.5)</td>
<td>1.58</td>
<td>0.465</td>
</tr>
<tr>
<td>ELISA vs. Cobas Integra 400</td>
<td>52.3 (5.7)</td>
<td>1.51</td>
<td>0.453</td>
</tr>
<tr>
<td>Cobas Integra 400 vs. Cobas e411</td>
<td>53.1 (5.8)</td>
<td>1.53</td>
<td>0.462</td>
</tr>
</tbody>
</table>

Results
For method comparison, a paired-sample T-test was used.
For consistency between methods, linear regression and correlation coefficient was used.
For determining accuracy, ROC curve was used.
Bias error between methods, Bland-Altman plot was used.
Statistical analysis was conducted at 95% confidence level.
P-value <0.05 was considered statistically significant.

Conclusions
Serum ferritin concentrations were measured by Cobas e411, and Cobas Integra 400 methods are strongly correlated with ELISA results with higher sensitivity, specificity, and accuracy.
Further investigations with larger samples are required for better accuracy and precision results and whether they can be used interchangeably.

Acknowledgments
Special thanks to Hadramout Modern Hospital in Yemen for technical support.
Special thanks to Laboratory Medicine Students for data collection and laboratory analysis performance.

Bibliography
Evaluating the Use of Non-High-Density Lipoprotein Cholesterol as a Superior Biomarker for Cardiovascular Risk Prediction: A Retrospective Cohort Study

Dena Nuaaifi1, Moto Gaddu and Zubair Awam2
1Clinical Biochemistry Department, Faculty of Medicine, University of Jeddah, Jeddah, KSA
2Clinical Biochemistry Department, Faculty of Medicine, King Abdulaziz University, Jeddah, KSA

Introduction

- Low-density lipoprotein cholesterol (LDL-C) is the primary treatment target in ASCVD (atherosclerotic cardiovascular disease) guidelines, despite its various limitations.
- Its major drawback is being self-reflective; it only represents the atherogenicity it retains.
- LDL-C has many other disadvantages, such as its fasting requirement if not directly measured.
- Other particles that carry a residual cardiovascular (CV) risk after LDL-C goal attainment include triglyceride-rich lipoproteins (TRLs) and Lp(a).

While non-high-density lipoprotein cholesterol (non-HDL-C) is a superior biomarker due to its ability to reflect all atherogenic apoB-containing particles, it was never used in clinical practice.

Non-HDL-C is also non-costly, does not require fasting, and a more reliable CV risk reflector in metabolically compromised states.

Research aims

- To validate the application of non-HDL-C as a better biomarker for CV risk prediction.
- To expand the knowledge of non-HDL-C impact on lowering CV risk if implemented in clinical practice.

Methodology

Study design and subjects’ selection

- A retrospective-cohort study using medical records from KAUH.
- 11,000 records were screened for baseline (untreated) lipid profiles from 2009-2016.
- Inclusion criteria - both genders, aged 18-85, with complete baseline lipid profiles.
- Exclusion criteria - patients treated for hyperlipidemia, labs tested on different dates, and death during follow-up.
- 800 subjects were enrolled, and followed up for CAD occurrence, of whom 446 were Saudis and non-Arabized separately, and 142 with normal lipid profiles.
- The median follow-up period was 8.5 years.

Data collection and biochemical measurements

- The following lipid biomarkers were obtained: TC, TG, LDL-C, and HDL-C. Non-HDL-C levels were calculated by: TC - HDL-C, while TRL-C by: TC - [LDL-C + HDL-C].
- All lipid parameters were directly measured, including LDL-C.
- TC, LDL-C, non-HDL-C, and TRL-C were compared for their ability to predict CV risk.

Results

Table 1: Characteristics of the study subjects grouped by CAD

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CAD (n = 446)</th>
<th>Non-CAD (n = 142)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60 (10)</td>
<td>56 (10)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.7 (0.9)</td>
<td>1.8 (1.3)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.2 (1.0)</td>
<td>3.2 (1.7)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.0 (0.5)</td>
<td>1.1 (0.5)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Non-HDL-C (mmol/L)</td>
<td>4.3 (1.5)</td>
<td>5.3 (1.8)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>TRL-C (mmol/L)</td>
<td>0.87 (0.76)</td>
<td>0.50 (0.52)</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Figure 1. Sensitivity analysis: ROC curves for the lipid biomarkers in (A) Multietnic, (B) Saudis

Figure 2. Relative risk (RR) of CAD associated with LDL-C and non-HDL-C in both populations

- The sensitivity of non-HDL-C for detecting cardiac events was the highest among all biomarkers in both populations (Figure 1).
- According to Spearmann’s correlation, non-HDL-C strongly correlated with TRL-C in those without CAD among multietnic, and in those with CAD only among Saudis.
- RR of CAD was higher with Non-HDL-C (Figure 2).

Discussion, conclusion, and recommendation

- Non-HDL-C had the highest diagnostic ability for CAD detection among all lipid biomarkers (Figure 1).
- The stronger correlation of non-HDL-C with TRL-C among those with CAD is anticipated given the commonness of hypertriglyceridermia risk factors in our society: obesity, MS, DM, and genetic susceptibility.
- High non-HDL-C was concurrent with more CAD incidence and a higher RR than with LDL-C in both populations (Figure 2).
- A multiple linear regression model adjusted for age, sex, BMI, HbA1C, and creatinine, predicted non-HDL-C to be 1.1 mmol/L higher than LDL-C among Saudis, which is higher than the 0.27 mmol/L difference that is recommended by guidelines, and is again foreseen in our population.
- Non-HDL-C was lower in most patients with CAD in the middle of the year, which is in line with seasonal variability.

Finally, we were able to demonstrate the value of non-HDL-C as a useful CV biomarker by addressing all apoB-containing lipoproteins, and we recommend further studies to support its employment in the management of hyperlipidemia for better cardiovascular outcomes.

References

The Assessment of Liver Function Test and Fertility Hormones In Athletes Using Anabolic Androgenic Steroids

Shaheer Jarral1,2*, Ammar Bani3, Rashed Khalid4, Abdullah Mangal5,6
1 King Abdullah International Medical Center, King Saud bin Abdel Aziz University for Health Sciences, Pathology, King Abdullah Medical City, Kingdom of Saudi Arabia
2 Department of Medical Laboratory Technology, Faculty of Applied Medical Science, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia

Introduction
A growing number of athletes are using synthetic anabolic-androgenic steroids (AAS), comprised of testosterone and other derivatives[1], to enhance athletic performance and muscle mass[2]. Over the years, numerous reports elucidated the side effects brought on by the illegal use of unsupervised AAS such as liver disorders including infertility and thrombotic complications, hepatic adenoma and hepatocellular carcinoma.[3] Consequently, AAS's recreational use has become a case of concern for the general public's health worldwide and should be brought to more serious attention worldwide and in particular Saudi Arabia. As of yet, AAS's effect on the hepatic and reproductive systems in Saudi athletes has never been studied[4]. Here, we examined liver function and sex hormone parameters of AAS users as compared to non-users.

Aim
To identify the effect of AAS on liver parameters (Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), gamma glutamyl transferase among (GGT), Alkaline Phosphatase (ALP) Total Bilirubin, Direct Bilirubin, Total Protein, and the effect on muscle enzymes, Creatinine Phosphokinase (CK) and Lactate Dehydrogenase (LDH)).

II. To assess level of fertility hormones, Follicular stimulating hormone (FSH), Luteinizing hormone (LH), total testosterone, Estradiol, and prolactin (PRL).

Method

- **Participants:** 16 Participants
- **Age:** 10 AAS user
- **Control:** 6 Control

**Exclusion criteria:**
- Male
- Teenagers (12-17 years)
- High BMI
- Age >50

**Inclusion criteria:**
- Clinical illness
- Therapeutic AAS user

**Blood Collection**

- **Normotrope Test:** (Spectrophotometer)
- Mass-Whitney (U-test)

- **Analysis:** CHI-SQUARE 2000, A 988

**Results**

**Figure B:** Comparison between liver function test among AAS users and Control.

- **Figure C:** Comparison between muscle enzymes CK and LDH among AAS users and Control.

- **Figure D:** Comparison between fertility hormones among AAS users and Control.

**Conclusion**

Long term recreational AAS use, outside the therapeutic frame induces biochemical unfavorable changes that may increase chances of liver damage and infertility in Saudi athletes.

**Limitation**

I. Small sample size.
II. Difficulty in recruiting participants.

**References**

I. Unpublished data.
II. Measure CK isoenzymes.
Diagnostic Comparison Between Cord Blood and Filter Paper for the Screening of Congenital Hypothyroidism

Sahar Almhar"1, Eman Alhabshe1, Saud Alhushe1, Michael Teleman1, Fara Badi2, Alshe Alhabshe1, Mohammad Majedia1, Abeer Beral1

1King Abdullah International Medical Research Center, King Saud University, Riyadh, Saudi Arabia
2King Abdulaziz University, Jeddah, Saudi Arabia

Background
The human brain requires thyroid hormones for its growth and development. Which are essential for intact neurologic functions especially during the first 2 years of life. Therefore, abnormalities in thyroid hormone levels may lead to severe neurocognitive and developmental consequences, one of which is intellectual disability (1). Persisting of congenital hypothyroidism in infants with low and very low birth weight is significantly high and is said to be around 1 in 400 cases, but in the case of full-term infants, it is 1 in 4000 cases (2). Therefore, screening programs and better management plans have been established to prevent and overcome this disease (2). Cord blood and heel prick TSH tests are utilized in diagnosing and preventing the severe complications of congenital hypothyroidism (1). The study aimed to compare between cord blood and heel prick TSH sensitivity and specificity in detecting congenital hypothyroidism (CH) among newborn screened babies in King Abdullah Medical City-Jeddah, Saudi Arabia.

Methods
The study included 21,452 newborn screened babies for congenital hypothyroidism during September 2014 until March 2016. Both cord blood and heel prick TSH were collected from each newborn. Heel prick and cord blood TSH cut-off values of >22 mIU/L and >50 mIU/L respectively were considered positive.

Laboratory methods:
For cord blood TSH was performed using 2000 Architect chemiluminescent immunoassay (Abbott Diagnostics) since 2013 until now. TSH measurement in heel prick sample using dry blood spot filter paper was performed using Generic Scree3 Processor by Perkin Elmer method.

Results
Flow chart showing the total number of neonates included in the study and the number of TSH positive samples collected by both heel prick and cord blood.

Conclusion
Cord blood TSH appears to be more practical option as a screening method for CH detection due to lower cost, lower workload, and instantaneous action. Especially in countries where the earliest possible discharge is their current practice, and it is challenging to get the newborn back to do the test in the hospital.

Recommendation
Healthcare centers who are looking for high sensitivity regardless of the recall rate can use the heel-prick as screening method for CH. Heel prick test is the superior screening choice for premature low birth weight babies.

Acknowledgement
The authors acknowledge support received from King Abdullah International Medical Research Center (KAIMRC), King Saud bin Abdulaziz University for Health Sciences (KSAU-HS), Jeddah, Saudi Arabia. The authors are also grateful to the hospital information system and medical records departments for their help and cooperation in providing data.

References
7th Annual Conference

30th Nov – 3rd Dec 2021