**وه‌زاره‌تی خوێندنی باڵا و تۆێژینه‌وه‌ی زانستی**

 **Ministry of Higher Education Scientific Research**

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| **پرۆپۆزەلى توێژینه‌وه‌ بۆ به‌ده‌ستهێنانی بروانامه‌ی دکتۆرا PhD Research Proposal**  |
| **ناونيشانی پرۆپۆزه‌لی تۆێژینه‌وه‌ی پێشنیازکراو 1. Title of PhD research proposal** **Role of Some physiological biomarkers and OX-LDL، BNP, ICAM-1 and** **IL-6 gene polymorphisms in ischemic heart disease** |
| **زانیاری گشتی 2. General information**  |
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| 3. Summary (Abstract) of PhD research proposalThis should be not more than 200 words and not less than 75 words.  ئەبستراکتی توێژینه‌وه‌ی پێشنیازکراو. له‌ 200 ووشه‌ زیاتر نه‌بێت و له‌ 75 ووشه‌ که‌متر نه‌بێت.  Cardiovascular diseases (CVDs) have been the prime cause of mortality worldwide for decades. However, the underlying mechanism of their pathogenesis is not fully clear yet. Various risk factors for CAD, including hypertension, diabetes mellitus, and hypercholesterolemia, have been identified epidemiologically based on their relations to the incidence of CAD (Shimokata et al., 2004). And it is the most common cause of death in the World. It results from a degenerative process with variety of arterial wall lesions. In this process, focal intimal thickening, following endothelial cell injury and proliferation of smooth muscle cells resulted in atherosclerosis. This process is accompanied by the participation of inflammatory cells (Nomani et al., 2011). It is a complex disease with the interplay of multiple genetic and environmental factors precipitating its development. (Z. Liang et al., 2015). Furthermore, Cardiovascular disease (CVD) is the leading cause of death and disability worldwide. Conventional risk factors for CVD, such as hypertension, diabetes mellitus, smoking, and hypercholesterolemia, have led to the development of risk prediction models and major developments in therapy. However, up to 20% of patients with coronary disease have no traditional risk factors, and 40% have only one (Wang et al., 2017).Atherosclerosis is a significant vascular disease globally, with clinical presentations such as ischemic heart disease (IHD), stroke, and peripheral arterial disease. The notion that atherosclerosis is a modern-day illness is incorrect. Mummies dating back to 3300 BCE have been shown to have atherosclerosis in all vascular beds, as well as in various geographic regions, cultures, and lifestyles at the time. {Thompson, 2013 #295;Zink, 2014 #294} Inflammation plays an important role in the pathogenesis of coronary heart disease (CHD). Several inflammatory cytokines have shown a direct association with the development of atherosclerosis {Ong, 2018 #296}.  |
| **4. Introduction پێشه‌کی**  To be completed by the primary supervisor: an overview of the proposed research project, focusing on the background of the project and rationale for the research.لێره‌دا سه‌رپه‌رشتیاری سەرەکی پوخته‌یه‌ک ده‌رباره‌ی پرۆژه‌ی توێژینه‌وه‌که ده‌نووسێت‌، تیایدا باکگراوندی پرۆژه‌که‌ باس ده‌کات و ڕوونی دەکاتەوە کە بۆچی ئاراستەکردنی ئەم توێژینەوەیە گرنگە. This project is more crucial of cardiovascular disease diagnosis and prevention, because today Physicians cannot diagnose cardiovascular disease using normal diagnostics; instead, they must request genetic tests ranging from sequencing a single gene or a panel of genes linked to the disease to unbiased whole-exome or whole-genome sequencing, which queries all genes.Induction myocardial infarction, for example, is caused by mutations in genes that code for atherogenesis and inflammation. Only patients with particular mutations will benefit from cardiovascular medicines, therefore special biomarkers and genetic testing for them can help guide treatment options. As a result, genetic testing is not only beneficial to patients but also cost effective.**Research Questions**The project presented here focuses on Myocardial Infarction and asks whether gene related to inflammation and atherosclerosis results from interactions between genetic and environmental factors. Would the gene polymorphism potentially modify the risk of CAD and can be diagnosis by special biomarkers?   |
| **5. Research objectives**Clarify the research objectives and planned methodology to meet the challenges of the project. Include details of the research plan and relate to the previous work carried out by others.لێره‌دا سه‌رپه‌رشتیار ده‌بێت ئامانجه‌کانی توێژینه‌وه‌که ڕوونبکاته‌وه و‌ باس له میتۆدەکانی ڕووبەرووبوونەوەی ئەو تەحەدیاتانە دەکات کە لەکاتی توێژینەوەدا دێتە ڕێگای، هەروەها گرنگە کە پلانی توێژینەوەکە ببەستێتەوە بەو کارانەی کە پیشتر لەو بوارەدا ئەنجام دراون.**The General Objective of the project** is to identify the association of genes that cause cardiovascular diseases (Angina Pectoris, Myocardial Infarction, Heart Failure, Stroke) in particular genes related to Atherosclerosis and inflammations. As well as those that cause heart disease regression which potentially leads to cure patients with Myocardial infarction.**Specific objectives of the project** To test gene related to Atherosclerosis, OX-LDL & BNP gene polymorphism and inflammations ICAM-1 and IL-6 gene polymorphism in patient samples. To determine the association of OX-LDL، BNP, ICAM-1 and IL-6 SNPs and gene sequencing with traditional and emerging biomarkers of CVD risk in Erbil city of patients with IHD, and to determine if some risk factors interact with these associations in this patient.‌ |
| 6. Methodology and data collectionIn this section the supervisor should describe the methodology of the proposed research لێرەدا سەرپەرشتیار باس لە میتۆدەکانی ئەنجامدانی توێژینەوەکە و شێوازی کۆکردنەوەی داتاکان دەکات.Subject Data Collection: The study comprises of collecting diagnosed CAD cases (n=100) in particular the ECG, Echo, angiography, biochemical and haematological screened tests. Including 30 healthy subjects as controls of matched age and sex, as well as using surveys to gather data about the subjects, a questionnaire would typically be used for this purpose. The control subjects will be diagnosed by a specialist (cardiologist), after making the necessary procedures to make sure that these subjects are healthy volunteers without established CAD complications. Together with recruiting the CAD patients from Erbil Cardiac Center. **Questionnaire Sheet**After the subjects were deemed appropriate to be included in the study, they were asked questions on that basis on a specially designed questionnaire, the questionnaire was designed was as follows:**Coronary Artery Disease (CD) Questionnaire**

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| Survey Questions |
| 1. Name:
2. Age (Years):
3. Phone No.:
4. Sex:  Male  Female
5. Nationality:  Iraqi  non-Iraqi
6. Occupation: Unemployed: Public Employed Private Employed Retired
7. Address: Urban Semi-urban Rural
 | 1. Marital status:  Single  Married Separated/ divorce
2. Income:  <1000 $ 1000- 2000 $ > 2000$.
3. Education:  Primary school  High school  University graduate  Msc/ PhD Illiterate.
4. Body Mass Index (BMI): (Height= , Weigh = ).
 |
| **Which of the following is a risk factor for coronary artery disease?**1. Smoking:  Yes  No  Ex-smoker2. Alcohol consumption:  Yes  No  Former drinker3. Lack of physical activity (at least 3 minutes of walking 5 days per week):  Yes  No4. Fast food intake:  Yes  No5. Soft drink intake:  Yes  No (Coca-Cola, Pepsi etc.)6. Hours spent weekly watching TV:  Do not watch  Less than 14 hours  more than 14 hours7. Hours spent weekly on computer:  Do not use  less than 14 hours  more than 14 hours8. Hours spent weekly on Cell phone:  Do not use  less than 14 hours  more than 14 hours9. Personal history of diabetes:  Yes  No10. Personal history of stroke:  Yes  No11. Personal history of heart attack diagnosed by a physician:  Yes  No12. Family history of diabetes:  Yes  No13. Family history of hypertension:  Yes  No14. Family history of hyperlipidemia (high cholesterol or triglycerides level):  Yes  No15. Family history of heart attack diagnosed by a physician:  Yes  No16. Family history of coronary artery disease diagnosed by a physician:  Yes  No17. DO you have social media  Yes  No  How many account do you have \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |

**Sample Collection**  **Blood and tissue Collection** Blood Collection: Collecting 10 ml of the venous blood sample from the antecubital vein of the CAD patients as well as from the healthy controls, under aseptic conditions. The blood sample will be divided into two halves: one in an EDTA vial and another in a plain vial to obtain serum. Then each one will be distributed into four Eppendorf tubes for ascertaining sample preserve purposes.**Serum Measurement:**All the samples must be screened for the oxidative and antioxidant parameters. Including the Malondialdehyde (MDA) biomarker of oxidative stress, tacrolimus (TAC) measure the amount of the drug in the blood, NO, Myeloperoxidase (MPO) measuring the antibody levels, superoxide dismutase (SOD) levels, Oxidised LDL (Ox-LDL).Equipment and Instruments1. Instruments and equipment
2. Autoclave
3. Cell sens 8.1 Dimension
4. Centrifuge
5. Cobas e411 Analyzer
6. Conventional PCR
7. Cryostate HM 500 M
8. Disposable Syringes
9. Dissection tools
10. Electrophoresis System
11. Eppendorf racks
12. Eppendorf tubes 1.5 mL
13. Gel documentation system
14. Micro Centrifuge
15. NanoDrop
16. OCT
17. PCR tube Racks
18. PCR tubes 0.2 mL
19. Pipette 0.1-2µl
20. Pipette 2-20µl
21. pipette100-1000µl
22. Precision balance PFB
23. Stereomicroscope SZX9
24. tips10µl
25. tips100µl
26. Vacuette Clot activator tubes
27. Vacuette EDTA tubes
28. Vortex
29. Water bath
30. Water Distiller

**DNA Extraction and Genotyping**.1. Absolute Ethanol
2. Agarose
3. Aqua-Mount mounting media
4. Distilled water
5. DNA Marker 100 bp
6. Formaldehyde 4%
7. Heamatoxylin
8. Phosphate buffer Tablet
9. Prime Safe Dye
10. Prime Taq Premix mastermix
11. PrimePrep Genomic DNA
12. DNA extraction kit
13. Primers
14. Runx2
15. Safe dye
16. Tris-Borate-EDTA Buffer

Genomic DNA Mini Kit content for100 samples**Table .** Genomic DNA kit was used in this study.

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| --- | --- | --- | --- |
|  **Component** |  |  **Amount** |  |
| Spin column |  | 50 pcs |
| Collection tube |  | 100pcs |
| Buffer GB |  | 12 ml |
| Buffer W1 |  | 20ml |
| Buffer W2 |  | 10ml |
| Buffer GE |  | 10ml |
| ProteinaseK solution(20mg/ml) |  | 1.2ml |

**Biochemical parameters** Biochemical parameters included: Serum GPT, GOT, urea, Creatinine, eGFR by Equation, Total Bil., Alp, LDH, Glucose, Cholesterol, TG , HDL, LDL, hs.CRP, CKmB, Troponin, And elisa parameters like MDA, Total Antioxidant capacity, SOD, GPx, Zinc Content Assay, oX-LDL, IL-6, Serum BNP, Serum ICAM-1, MPV and platelet count were recorded and evaluated. The assay was performed by e411 cobas, (Roche HITACHI, Germany). Clinical laboratory indexes included platelet count and MPV were also measured to estimate the rate and concentration of blood using coulter counter.**Cardiac Measurements**Twelve-lead electrocardiograms were recorded in all participants using standardized proceduresQRS, Duration, ST (Elevation or Depression), Sinus rhythm and T-wave inversion**Cardiac Angiography****DNA extraction** DNA from the blood was extracted according to the protocol of Primeprep Genomic DNA extraction Kit/Korea. Twenty microliter of proteinase K was added to 1.5ml tube then 200μL of whole blood sample mixed with it. Two hundred microliter of GB buffer was added and mixed well by vortex. The samples were incubated at 56C° around 10 minutes. After incubation 200μL of Absolute ethanol was added with well pulse vortexing. Then transfer the lysate to the spin column. The samples were centrifuged at 10,000 rpm for 1 minute. Five hundred microliter of GW1 buffer was added then centrifuged at 10,000 rpm for 1 minute.The flow through was discarded and transferd the spin column to new collection tube. The excess ethanol was removes by more centrifugation at 12,000 rpm for 1 to 2 minutes. Two hundred microliter of GE buffer was added and incubated at room temperature for 1 minute.Eventually DNA was eluted by centrifugation 10,000 rpm for 1 minute. The extracted DNA samples were stored at -20C°. **DNA quantification determination**The quantity of isolated DNA was measured using NanoDrop™ 1000 Spectrophotometer. The Nanodrop instrument was calibrated and washed using distilled water prior measuring of samples and dried the blank solution from the upper and lower pedestal.1 µL of samples was loaded to the lower micro volume pedestal. Spectrophotometric analysis is depended on the basics that DNA absorbs ultraviolet light in a specific pattern. The samples were stored at -20°C until the time of use.**DNA Amplification****Preparing the primers**The pre-designed primers were directly ordered from Integrated DNA technology (USA). Upon their arrival, the lyophilized primers were re-suspended by nuclease free water according to primer protocol to prepare a 100 pmol stock solution primer. The working primers were prepared by mixing 10 μL of stock primer with 90 μL of Dnase, Rnase free water.**Preparing the samples for PCR amplifications**The master mix (Prime Taq Premix master mix) solution is ready to use for PCR amplification, having Taq DNA polymerase, Deoxynuceotide triphosphates (dNTPs), magnesium chloride (MgCl2), KCl and Tris HCl, and reaction buffer at optimal concentration for efficient amplification of DNA template by PCR. It contains all requirements for amplification reaction in thermal cycler, beyond DNA template and primers. The reagents required for PCR were mixed in a sterile nuclease free (0.2ml) Eppendorf tube as shown in the following Table 3.5. **Table 3.5.** Ingredients of PCR solutions required for master mix reaction preparation of one sample (20 µL) for PCR.

|  |  |
| --- | --- |
| **Reaction mixture** | **Amount** |
| Prime Taq Premix mastermix | 10µL |
| Forward primer | 1µL |
| Reverse primer | 1µL |
| Common primer | 1µL |
| DNA template | 3µL |
| Nuclease free water | 4µL |
| Total | 20µL |

In this study, 100 samples were analyzed. **10**μL of mastermixture was added into 0.2 ml Eppendorf tube, 1μL for each primer, 3μL of DNA of each sample was added to these tubes individually. The volume was completed to 20μL with nuclease free water and mixed gently. The PCR running was done by to round, first round all samples were checked by using T forward primer with common primer. In the second round all DNA samples were amplified by using C forward primer with common primer. The tubes were positioned in the thermal cycler to carry out amplification according to PCR program in Table3.6. For the OX-LDL, IL-6, ICM1, BNP genes, we used two sets of primers to determine the polymorphism because the polymorphism occurs at the exact location, where the differences in the primer sequences are seen. **Table 3.6** the amplification program.

|  |  |  |  |
| --- | --- | --- | --- |
| **Steps** | **Temperature (°C)** | **Time** | **No. of cycles** |
| Initial denaturation | 95 °C  | 5min | 1 |
| Denaturation | 95 °C | 30sec | 35 |
| Annealing | 64.2 °C | 50sec |
| Extension | 72 °C | 1min 30s |
| Final extension | 72 °C | 5 min | 1 |

**Agarose Gel Electrophoresis Preparing and Running****Agarose Gel Preparation Method**A glass board with suitable dimension of gel electrophoresis tank was prepared. The edges of board were surrounded by strong tip in which a special comb was fixed to make wells in one side of gel. Agarose was made in 2% by dissolving 2g of agarose in 10 ml of 10X TBE buffer and the volume was completed to 100 mL of distilled water, and boiled continuously with striking, and then left to be cooled to 50-55 C°. Subsequently 15μL of safe dye was added to visualize DNA bands. The formula was poured gently, continuously and quietly and left to be solid. The tip and comb were removed quietly, and the gel was immersed in 1X TBE solution. The gel was put in electrophoresis tank that contained 1X TBE buffer. Then 10μL of each PCR product sample was loaded gently into the agarose gel well, avoiding spillover to adjacent wells. A first lane was always left for loading a 100 bp Ladder DNA (Norgenbiotek/ Canada).**Gel Electrophoresis Running**The lid of the gel apparatus with its adjoining electrodes were attached making sure that the negative one was on the same side as the wells. For a better resolution power supply of 45 volts was applied for 10 minutes, until the DNA left the wells and moved above 0.5 cm towards the positive electrode. Then the voltage was increased to 75 volts and the electrophoresis was allowed to proceed for a sufficient time (usually 30 min).**Visualizing the genotypes**After the samples were run on the gel, DNA bands were visualized by the presence of safe dye that binds with DNA strands and the gel was documented by using Gel documentation system (Proxima isogen life science, Netherlands) for visualizing and analyzing DNA bands. The machine was connected with a computer for capturing the images of the DNA band and the brightness and contrast were controlled in the software. |
| **7. Scope and limit to the research** Details of anticipated problems and proposed resolutionsلێرەدا باس لەو بەربەستانە دەکرێت کە دەشێت بێنە ڕێگای ئەنجامدانی توێژینەوەکە، هەروەها باس لە چارەسەری ئەو بەربەستانەش دەکرێت. 1. Real time PCR
2. Buying chemical and Elisa Kits
3. Sequencing DNA
 |
| **8. Duration and timeline**لێرەدا باس لە کاتی پێویست بۆ ئەنجامدانی توێژینەوەکە دەکرێت Here we need two years to completing the project  |
| **9. Conclusions**The project supervisor summaries the research objectives and clarify their expected findings; include why the research has scientific value. لێرەدا سەرپەرشتیار باس لە گرنگی ئامانج و دەرئەنجامە چاوەڕوانکراوەکانی توێژینەوەکە دەکات، هەروەها ڕوونی دەکاتەوە کە بۆچی ئاکامەکانی ئەم توێژینەوەیە بەهای زانستیی هەیە.  |
| **10. References** سەرچاوەکان **1**.Sanchis-Gomar F, Perez-Quilis C, Leischik R, Lucia A. Epidemiology of coronary heart disease and acute coronary syndrome. Ann Transl Med. 2016;13:256. **2.** Jourdain P, Jondeau G, Funck F, et al. Plasma brain natriuretic peptideguidedtherapy to improve outcome in heart failure: the STARS-BNP Multicenter Study. *J Am Coll Cardiol*. 2007;49(16):1733-1739.**3.** Pfisterer M, Buser P, Rickli H, et al. BNP-guided vs symptom-guided heart failure therapy: the Trial of Intensified vs standard Medical therapy in Elderly patients with Congestive Heart Failure (TIME-CHF) randomized trial. *JAMA*. 2009;301(4):383-392.**4.** Lainchbury JG, Troughton RW, Strangman KM, et al. N-terminal pro-Btype natriuretic peptide-guided treatment for chronic heart failure: results from the BATTLESCARRED (NT-proBNP-Assisted Treatment To Lessen Serial Cardiac Readmissions and Death) trial. *J Am Coll Cardiol*. 2009;55(1):53-60.**5.** Zacho J, Tybjaerg-Hansen A, Jensen JS, Grande P, Sillesen H, NordestgaardBG. Genetically elevated C-reactive protein and ischemic vascular disease.*N Engl J Med*. 2008;359(18):1897-1908.**6**-Di Pietro, N., Formoso, G. & Pandolfi, A. Physiology and pathophysiology of oxLDL uptake by vascular wall cells in atherosclerosis. *Vascul. Pharmacol.* **84**, 1-7, https://doi.org/10.1016/j.vph.2016.05.013 (2016).**7**. Smiljić, S., Mijović, M. & Savić, S. Biomarkers of endothelial dysfunction in cardiovascular diseases. *Med. Pregl.* **70**, 53-57, https://doi.org/10.2298/MPNS1702053S (2017).**8.** Li, D., Qu, C. & Dong, P. The ICAM-1 K469E polymorphism is associated with the risk of coronary artery disease: a meta-analysis. *Coron. Artery Dis.* **25**, 665-670, (2014).**9.** Wang, D. *et al*. Association of polymorphism in ICAM-1 (K469E) and cytology parameters in patients’ initial blood test with acute ischemic stroke. *Genet. Mol. Res.* **14**, 15520-15529, (2015).**10.** Chou, C. H. *et al*. Impact of intercellular adhesion molecule-1 genetic polymorphisms on coronary artery disease susceptibility in Taiwanese subjects. *Int. J. Med. Sci.* **12**, 510-516, (2015).**11**. Yin, D. L. *et al*. Association between the ICAM-1 gene polymorphism and coronary heart disease risk: a metaanalysis. *Biosci. Rep.* **39**, BSR20180923, (2019). |
| **11. General notes:** هەر زانیارییەکی گشتی دیکە کە سەرپەرشتیار بە گرنگی بزانێت |
| **12.** **په‌سه‌ندكردنی پرۆپۆزەل له‌ لایه‌ن لیژنه‌ی زانستی به‌ش**ژماره‌ی كۆنووسی كۆبوونه‌وه‌:رێكه‌وتی كۆبوونه‌وه‌:بریار: په‌سه‌ند كرا په‌سه‌ند نه‌كرا  ناوی سیانی و واژووی لیژنه‌ی زانستی به‌ش واژوو:  ناوى سه‌رۆكی لیژنەى‌ زانستی به‌ش مۆری به‌ش  واژوو:  ناوى سه‌رۆكی به‌ش:  |
| **13.****په‌سه‌ندكردنی پرۆپۆزەل له‌ لایه‌ن ئه‌نجومه‌نی كۆلێژ/فاکەڵتى**ژماره‌ی كۆنوسی كۆبوونه‌وه‌:رێكه‌وتی كۆبوونه‌وه‌:بریار: په‌سه‌ند كرا په‌سه‌ند نه‌كرا  واژوو: ناو راگری كۆلێژ: مۆری كۆلێژ |

**تێبینی:** تكایه‌ فۆرمه‌كه‌ ته‌نها به‌ یه‌ك زمان (زمانی توێژینه‌وه‌) پڕ بكرێته‌وه‌.