MELVIN ALFRED TOKPAH

GENE EXPRESSION PROFILE OF INTERLEUKIN 8 & INTERLEUKIN 18 PATHWAYS IN VEINS ABD AORTA ANDVARIOTA

M.Sc. THESIS

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May, 2023

Approval

We certify that we have read the thesis submitted by **MELVIN ALFRED TOKPAH** titled **"GENE EXPRESSION PROFILE OF INTERLEUKINS 8 & INTERLEUKIN 18 IN VEINS AND AORTA"** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science in Biochemistry.

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DECLARATION

I hereby declare that all of the information contained in this paper was gathered and presented in a manner that is compliant with academic regulations and ethical standards of behavior. I further declare that, in accordance with these rules and standards of behavior, I have properly attributed and referenced all information and outcomes that are not my own and are not unique to this work.

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Melvin Alfred Tokpah

Abstract

The Expression Profile of Interleukin 8 & Interleukin 18 Pathways in Veins and Aorta

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AIM:

The goal of the research was to analyse the expression pathways of *IL-8* and *IL-18* genes in veins as well as in aorta tissues that were obtained from patients suffering from a wide range of cardiovascular conditions.

BACKGROUND:

Interleukins, also known as chemokines, are an important component of our system of immunity because they are responsible for regulating the path that leukocytes take during movement and for stimulating the cells that are responsible for the immune response. *IL*-8 is the human chemokine that attracts neutrophils more effectively than any other and performs important functions in the body's response to infections and injuries to tissues. Interleukins gene is expressed differently in various tissues and specimens, understanding how effective the gene is express in body tissue provides a blue print for further studies.

Numerous studies have been conducted to understand the expression of interleukins and their roles using different body specimens including blood, cardia muscles of rats but there are limited report on the use of human veins and aorta tissues to understand the expression pathways of interleukins.

Understanding the expression pathways of interleukins 8 and interleukin *18* is very important because of their implication in various disease conditions including cardiovascular disease.

Cardiovascular diseases (CVDs) are a category of illnesses that have an impact on the circulatory system and blood vessels. Heart attacks and strokes are responsible for more than four out of every five cardiac deaths, and people under the age of 70 account for one-third of those deaths.

METHOD:

For the purpose of this investigation, samples of human aortas and veins were collected from the IVF center and laboratory at the "Near East University Hospital (NEUH)". After receiving permission from the scientific review board of NEU, 103 samples of veins and aorta were taken from patients who were diagnosed with cardia vascular diseases. These samples were categorized as either the patient or control group, the control group for vein were those with normal veins.

The Molecular Medicine Laboratory at the DESAM Institute of Near East University was the location where this research was carried out.

After RNA had been extracted, its concentration and purity were measured using a device called a Nanodrop; cDNA synthesis then followed, which was completed using a Transcription first-strand cDNA synthesis kit. The 96 wells bio systems thermal cycler PCR was utilized in order to carry out the gradient polymerase chain reaction.

After that," real-time RT-qPCR" was carried out using "Rotar Gene-Real Time PCR". In order to ensure accurate detection and quantification of the products that are created after each cycle of the PCR process. In the end, they were separated on agarose gel electrophoresis at a 2.4%w/v concentration.

RESULTS:

For the purpose of this investigation, tissue samples from the veins and aorta of 103 individuals who had been diagnosed with a variety of cardiovascular conditions were collected. These samples were examined to determine the levels of expression pathways of *interleukins 8* and *interleukin 18*.

Samples were taken in two separate batches and assigned to either the control group or the patient group. In the patient group, a total of 32 vein and 32 aorta samples were taken, which adds up to 64 patients of the samples. In the control group, on the other hand, 11 veins and 28 control samples were taken, which brings the total number of control samples to 39. The samples taken from the veins accounted for 43% of the total samples collected, whereas the samples taken from the aorta accounted for 60% of the samples taken.

We found that the expression of serum *IL-18* was statistically significantly higher in control veins than the control aorta, with a P-value of 0.0003. With a P-value of 0.0151, the *IL-18* concentrations found in the vein of the patient group were

statistically higher than the *IL-18* expression in the aorta of the patient groups additionally, *IL-18* is also more expressed in the aorta of the control group than the patient group as shown in table 3.3.

In addition, the research results demonstrate that the *IL*-eighteen expression in the veins of patient is significantly lower than the expression of *IL*-eighteen in the veins of the control group, with a P-value of 0.0008.

IL-eight expression in aorta tissues of the control study group is statistically significantly greater than the level of *IL-eight* expression in vein tissues of the control study group, as indicated by a P-value of 0.0158.

A significant statistical difference was observed between the *IL*-eight expression of patient aorta and *IL-eighteen* of the control veins with a P-value of 0.0015. And more over the "*IL-eight* expression" level in the patient study group's vein tissue was statistically substantially lower than the *IL*-8 expression level in the patient study group of aorta tissue.

CONCLUSION:

According to the results obtained by this research so far, *interleukin- eight* and *interleukin eighteen* were both significantly expressed in the aorta tissue of both the control group and the study group of patients. In contrast to *interleukin-eight*, the expression of *interleukin-eighteen* was predominately expressed in both the veins and aorta tissues. Because of this, *interleukin-eighteen* is considered a primary biomarker, and either a lack of expression or an excess of expression makes an individual more likely to experience essential underlying health concerns.

In conclusion, the exact place that *interleukin-eighteen* occupies in the functional hierarchy of pro-inflammatory cytokines during chronic inflammation is not entirely clear, despite the fact that there is widespread agreement that it plays an important role early on. However, the processes of synthesis, regulation, and subsequent release of *interleukin-eighteen* are not well understood, even though the fact that *interleukin-eighteen* plays a role in both host defence and inflammation.

In a similar vein, the modulation of *IL-18* bioactivities in vivo in the context of high levels of *IL*-18BP and other native inhibitors is still in need of further explanation. In addition, the findings of this research indicate that the aortic tissues are an excellent source of *IL*-eighteen expression.

This finding suggests that the aorta tissues, in addition to blood, plasma, and serum specimens, might be utilized to better understand how *IL*18 is expressed and regulated in the body.

Since *interleukin* screening affects the population at all levels, including pregnant women, children, and the elderly, and is also a major contributing factor and biomarker for several biological activities and illnesses, it is important that it play a pivotal role in the health system. *IL* screening should be consider one of the primary functions of the health the system.

Keywords**:** gene expression, interleukin-8, interleukin-18, veins, aorta, cardiavascular disease

Contents

LIST OF ABBREVIATION

µl: Microliter

µM: Micro molar

nM: Nanomolar

bp: Base pair

β: Beta

DM: Diabetes mellitus

NIH: National institute of health

cDNA: Complementary deoxyribonucleic acid

RNA: Ribonucleic acid

PCR: Polymerase chain reaction

qRT- PCR: Quantitative reverse transcriptase – polymerase chain reaction

NTC: No- template control

Ct: Cycle threshold

CVDs: Cardiovascular Diseases

EDCs: Endocrine disturbing chemicals

SNP: Single nucleotide polymorphism

DHEAS: Dehydroepiandrosterone sulfate

LRP: Lipoprotein receptor- related protein

RYK 7: Atypical receptor related tyrosine kinase

PTK7: Protein tyrosine kinase7

ROR2: Receptor tyrosine like orphan receptor 2

TBE: Tris borate EDTA.

CHAPTER I:

Introduction

This chapter includes the problems, aims, importance, limitations and related descriptions of the research.

1.0. Introduction

It is evident that *interleukins* or the *cytokine* play a role in human heart attacks, heart failure, and possibly numerous other types of heart disease (Ji et al., 2021). It was discovered that the atherosclerosis plaques that were removed during carotid endarterectomy in humans had greater concentrations of *Interleukin-18* (IL-18) expression, and the accumulation of this protein has been linked to plaque destabilization.

Ferrer-Gómez et al., (2021) evidence suggests that "inflammation," in addition to related *"interleukins*" like *IL-1* and *IL-18*, could have a substantial impact on the tissue damage responses that "myocarditis" generates. This is the case because "myocarditis" causes inflammation of the heart's muscle tissue. When injured tissue is present, "extracellular neutrophil proteinases" like "neutrophil proteinase 3 (PR3)" can activate *IL-18 (*Dubyak et al., 2023).

Even though most "*IL-18* is soluble", there is a chance that certain macrophages may contain it in a membrane-bound state. When lipopolysaccharide activates these cells, they release soluble *IL-18* (LPS). This suggests that an additional" LPS-induced protease", most likely PR3, may release *"IL-18* "and activate it." *Pro-IL-1* and *pro-IL-18* "produced by dying cells may be activated by PR3, in accordance with some ideas, according to (Yasuda et al., 2019) this determination was reached in light of the findings of a recent study.

It has been shown that the *Interleukin-18 (IL-8)* gene, as well as *CXCR1* and *CXCR2* genes, are more active in people with persistent perfusion defects (Leonard et al., 2011). It may be difficult for the LV to fill completely in patients who have heart failure but have preserved ejection fraction (HFPEF) because of increasing fibrosis and cardio myocyte hypertrophy. This condition is abbreviated as HFPEF (Quarto, 2020; Muñoz, 2017). Since it plays a part in the pathogenesis of the condition, *IL-18*

is a candidate for being a target for “HFPEF due to the PR hypertrophic and profibrotic” actions that it carries out.

1.1. Interleukins

“*Interleukin” (IL),* a type of cytokine, was once thought to be made only by leukocytes, but it has since been found that many other types of body cells also make it. They are crucial for the development, maturation, mobility, and adhesion of immune cells, as well as for their activation and differentiation. They also possess both “pro- and anti-inflammatory” qualities (Gillis, 2013). White blood cells, as well as numerous other cells all over the body, express and secrete "interleukins," a particular type of "cytokine" (in the form of secreted proteins and signal molecules).

1.1.1. *Functions of Interleukins*

Thus, controlling proliferation, development, and stimulation is the primary function of interleukins in immunological and inflammatory responses. Interleukins are composed of numerous protein molecules that attach to cell surfaces with strong affinity. This results in a range of diverse reactions in the cells and tissues. In addition to their paracrine function, they also have an autocrine one. Interleukins are also used in animal studies to look into different areas of clinical medicine (Vaillant et al., 2022).

1.1.2. *Defence of the IL provided by the host and the mediator*

It has been discovered that patients suffering from a variety of allergic disorders have elevated amounts of the "pro-inflammatory cytokine *IL-18"* circulating in their blood. Some of the disorders that fall into this category are “bronchial asthma”, "atopic dermatitis," and allergic rhinitis. *“Interleukin- eighteen* is a powerful "pro-inflammatory cytokine" that governs both the "inherent" and the "acquired" immune responses. Additionally, it assists the host in warding off infections by playing a role in the immune system (Paparazzo et al., 2022). In addition to macrophages, other types of cells are also have the potential to produce the cytokine known as "IL-18." This "cytokine" is a member of the "interleukin one superfamily" and has actions that are both "pleiotropic" and capable of activating an extensive variety of cells. *"Interleukin eighteen"* is a cytokine" that is known to promote inflammation and boost immunological responses of type 1.

As a result of exposure to microbial compounds, such as "lipopolysaccharide." (LPS), it is also responsible for triggering Cell-Mediated Immunity in the body along with "*IL-12*" (Paparazzo et al., 2022). When "*IL-18"* and "*IL-12"* interact with "CD4"

and "CD8," "T cells," as well as " natural killer cells," the production of “IFN”, a type II interferon” that is necessary for activating “macrophages” and other cells, occurs. IFN is required for activating macrophages and other cells.

It has been demonstrated that the combination of *"IL-18 and IL-12"* is able to inhibit the formation of *"IL-4-dependent*" IgE and IgG1 in B cells, while at the same time boosting their production of "IgG2a" (Macleod et al., 2021). In the absence of *"IL-12 or IL-15, IL-18"* does not enhance the production of “IFN”; nevertheless, it does play a significant role in activating macrophages and other cells. This is an important point to keep in mind. It has been proven that the combination of *IL-18 and IL-12* has the ability to limit the manufacture of *"IL-4-dependent IgE*" and "IgG1 in B cells" while simultaneously enhancing their production of "IgG2a." (Gosmann et al., 2014). The principal cells that *"IL-8"* is directed toward are called "neutrophil granulocytes." However, this chemokine has an influence on a wide array of cell types, comprising "endothelial cells, macrophages, mast cells, and keratinocytes." Tetrahymena pyriformis was found to produce quantities of the chemoattractant *"IL-8"* that are comparable to those seen in vertebrates. This research demonstrated that *"IL-8"* is capable of acting as a “chemoattractant”. This demonstrates that both the structure and the function of this chemokine have been fully understood.

1.2. Interleukin 8 (IL-8), chemokine (C-X-C motif)

Many different types of cells in tissues and blood produce *interleukin-8 (IL-8)* as a cytokine (Islam et al., 2022). Unlike most other cytokines, this one has a clear target specificity for the neutrophil, and it has very little effect on the other blood cells. Interleukin-8 attracts neutrophils like a magnet and makes them work harder in places where there is inflammation (Matsushima et al., 2022). The “pro-inflammatory cytokine” known as “*interleukin eight* has significant effect on the immune cells that make up the body, including polymorph nuclear cells (Marino et al., 2020).

Keratinocytes are an abundant source of *“interleukin-eight (IL-8)”. Interleukin eight* is a cytokine that is a member of the “CXC” chemokine family. It is also known as “neutrophil activating peptide, CXCL8”, small inducible cytokine subfamily B (SCY88), and granulocyte chemotactic protein-1 (GCP-1). The *“IL-8 gene*”, which is located on chromosome “4q12-q21” encodes the small protein known as *IL-8* (Jiang et al., 2012). Interleukin- eight is a molecule that controls immunological reactions (Bharati, 2022).

Many disorders, including “viral bronchiolitis”, “cystic fibrosis”-related lung pathology, infection, and other inflammatory diseases such “rheumatoid arthritis”, have been associated with “interleukin eight”(Mannion et al., 2022). In the context of the pathophysiology of sepsis, it is a central player.

The *IL-8R* receptor, which is responsible for mediating *“IL-8”* action. “*IL-8”* is a potent “chemoattractant for neutrophils, basophils, and T cells but not for monocytes”, despite the fact that monocytes are a rich source of IL-*8”. “IL-8R”* comes in two different variations: *“IL-8RA and IL-8RB*”. “Chemokine, CXC motif, receptor-1 (CXCR1)”, and *IL-8R1* are all names that have been used to refer to *“IL-8RA*”. The location of the “*IL-8RA*” gene is on “chromosome 2q35” (Jiang et al., 2012). The gene that codes for *IL-8RB* can also be referred to as a “chemokine, CXC motif”, “receptor-2 (CXCR2)”, /“IL-8R2”. (Dhayni et al., 2022). This gene can be found on “chromosome 2q35”. Rich sources of *IL-8* can be found throughout the body in monocytes and fibroblasts (Larson et al., 2021).

A study by Jiang et al. (2012) indicated that therapy with Lactobacillus plantarum accelerated the healing of both "diabetic" and "non-diabetic leg ulcers". It expedited the healing so much that it may have had something to do with the rise in *"IL-8* production by "polymorph nuclear" cells in the wound base. In addition to this, it has been established that *IL-8* impacts the movement of both "fibroblasts and endothelial cells".

1.2.1. The function of Interleukin 8

Studies have demonstrated that the *interleukin 8* and its related receptors, "C-X-C chemokine receptor-1(CXCR1)" and "C-X-C chemokine receptor 2 (CXCR2)," are implicated in the development and progression of a number of various cancers (Liu et al., 2016). “CXCL8” also interacts with a number of intracellular signaling pathways so that its effects work together (Asokan & Bandapalli 2021). Moreover, it is well known that IL-8 is a strong inducer of angiogenesis. The physiological effects of “*IL-8”* on target cells include an increase in “intracellular Ca_2^+, exocytosis” and release of “histamine”, to name a few. These processes are essential for phagocytosis and motility (Perna et al., 2021). Any cell in the body that has toll-like receptors and participates in the immune system's natural response can secrete the cytokine known as *"IL-8"* (Pekalski et al., 2016). “CXCL8” is essential for initiating the cell signaling that is necessary to bring about the alterations that are needed, and this is so that

neutrophils can navigate their way through the basement membrane and the extracellular matrix (ECM) to reach the affected area (Cambier et al., 2023). When a neutrophil moves along the “endothelium”, it comes into contact with a “CXCL8” molecule that is released on the surface This brings about activation of the cell signalling pathway via a “G-protein-coupled receptor” (Cesta et al., 2022). The production of the integrin known as *"LFA-1"* increases when *"CXCL8*" attaches itself to *"CXCR1/2"* on "neutrophils." This integrin is a participant in high-affinity binding with*” ICAM-1 receptors*” that are “expressed” on the “endothelium”.*“LFA-1* expression” and “affinity” are significantly increased in order to achieve maximum binding, Because of this, the neutrophil's rate of movement is slowed even further, and eventually it comes to a complete stop. The beginning of an oxidative burst is another significant function that is triggered by *CXCL8*-stimulated cell signalling. This mechanism makes it possible for “proteolytic enzymes” and “reactive oxygen species (ROS)” to accumulate, both of which are necessary for the breaking down of the “extracellular matrix” and the under laying membrane (Mittal et al., 2014). These, along with additional integrins, are secreted in granules that are responsible for the production of secretions. Even though generation of reactive “oxygen species (ROS)” and enzymes that are harmful to the host is controlled to a certain degree in order to reduce the amount of damage done to the host, effector activities are still carried out at the site of the infection (Khan et al., 2023). It is believed that *IL-8* is involved in the etiology of bronchiolitis, which is frequent infection of the nasal passages that is brought on by a virus. The genes responsible for *IL-8* and the other eleven “CXC chemokine” family members are found in a cluster on “chromosome 4q (Rajabi, 2022; Matsushima, 2022).

1.2.2. CXCL-8 mediated chemotaxis of the neutrophil

Macrophages, in addition to other types of cells including epithelial cells, smooth muscle cells, and endothelial cells, are also in the airways and are responsible for the production of the *chemokine interleukin 8* (Hedges et al., 2000). Endothelial cells have their own storage vesicles, which are referred to as "Weibel-Palade bodies." Within these "Weibel-Palade bodies, "*IL-8"* is stored in preparation for later use (Wolff, 1998; Utgaard, 1998).

It was reported by Modi et al., (1990) that the “*CXCL8* gene” is responsible for encoding the “interleukin-8 protein” in human beings. *“IL-8”* begins its life as a

precursor peptide consisting of ninety nine amino acids. This peptide goes through cleavage, which results in the production of several active *IL-8* isoforms (Brat et al., 2005). When macrophages are grown in culture, the predominant form that they secrete is a peptide containing seventy-two amino acids (Brat et al., 2005). Chemotaxis is the term used to describe the movement of neutrophils toward an area that has been damaged or infected, and "*CXCL8* is the main cytokine" that is associated with this process (De Oliveira et al., 2016). One of the key elements in the successful chemotaxis of neutrophils is their capacity to make their passage through the "extracellular matrix (ECM)" and the "basement membrane" in order to get to the damaged tissue. "Neutrophils" are able to pass through the "extracellular matrix (ECM)" and the "basement membrane" in order to get to the damaged tissue, which is a key part of their ability. Inducing cell signaling that is required to bring about these changes is primarily the responsibility of *CXCL8*, which plays a pivotal role in this process (Dixit & Simon, 2012). The histamine that is generated in the infected area widens the capillaries close to the wounded area, this reduces the flow of blood to the area and stimulates "leukocytes, such as neutrophils", to move away from the lumen's center, where blood flow is at its maximum rate, and toward the endothelium. Second, the discharged of histamine at the infected area enhances the synthesis of "cytokines" that cause inflammation (Arlati, 2019). The expression of this protein is also increased as a result of the impacts of *"CXCL8*" and numerous other "cytokines". Following this, interactions between the selectins produced by endothelial cells and the selectins produced by neutrophils will become minimal, they are known as "L selectins on neutrophils and P and E selectins on endothelial" cells, respectively. This results in a stage of chemotaxis known as the "moving or rolling" stage (Arnau, 2021; Starckx, 2002).The neutrophil encounters the *"CXCL8"* molecule on the surface of the "endothelium" as it travels through the "endothelium". A G-coupled protein receptor is responsible for mediating the cell signalling pathway, which this interaction will stimulate (Campbell, 2021). When *"CXCL8* binds to *CXCR1/2* "on a "neutrophil, the neutrophil" is stimulated, as a result, the "neutrophil" raises the level of production of the integrin "LFA-1". This integrin is responsible for the purpose of elevated interfacing with endothelial "ICAM-1 receptors" (Combes et al., 2020). "G protein-coupled serpentine receptors" *CXCR1* and "*CXCR2"* are the most thoroughly studied receptors on the surface of the cell (Avazi et al., 2019). There are various surface membrane receptors that has the potential of connecting to *"IL-8*. *IL-8* expresses"

more and has a stronger affinity for the "*CXCR1* receptor" than the "*CXCR2* receptor". "*IL-8"* plays a crucial role as an immune response mediator in the body's natural defence mechanism and is released as a result of a number of metabolic processes. "Chemotactic factors" are released by both resident inflammatory cells and structural cells, like "epithelial" cells, and are responsible for both the increased flow of neutrophils and inflammation in the lungs (caused by macrophages) (Moldoveanu et al.,2008). Some of the most important "neutrophilic" chemo-attractants, "CXC chemokines," such as interleukin-8 *(CXCL8),* were identified in the "Broncho alveolar lavage fluid (BALF)" of people with COPD.

Therefore, for an effective host defence, the immune response caused by neutrophils needs to be regulated properly by the body, on the other hand, the bystander effect is linked to things going wrong with these cells and or processes (Trivedi et.al. (2012). *"CXCL8, IL-1B, TNF-α* and *LTB4"* are some of the mediators that have an impact on neutrophil recruitment to the airways (Gernez et al., 2010).

1.2.3. The clinical significance of interleukin 8 and its effect on metabolic homeostasis

Interleukin-8 is an important mediator that is linked to inflammation. Within this context, it plays an important part in the processes of neutrophil breakdown and activation. (Harada et al., 1994). *Interleukin-8* has been described "pro-inflammatory mechanism" in psoriasis and gingivitis, for instance (Che et al., 2022). Oxidative stress makes the body make more interleukin-8, which brings in more inflammatory cells and causes even more oxidative stress mediators to be made. This makes interleukin-8 an important part of the inflammatory response (Vlahopoulos et al., 1999). A link between obesity and *IL-8*, which is a localized factor or parameter of inflammation has also been described by (Sharabiani et al., 2011). *Interleukin-8 (IL-8)* is an essential variable in how malignant pleural mesothelioma becomes resistant to chemotherapy by causing the expression of transmembrane transporters, and it might also have an integral part in Colo-rectal cancer by acting as an autocrine growth factor for the colon "carcinoma" cell line (Cui, 2023; Itoh , 2005; Milosevic ,2020).

According to Fan et al. (2022), a mother's *interleukin-8* levels during pregnancy can affect the likelihood that her child will develop "schizophrenia". Krigbaum et al., (2019) findings also demonstrate a connection between elevated maternal inflammation during pregnancy and infancy, increasing the chance of developing a

number of mental problems later in life. “Schizophrenia” susceptibility has repeatedly been associated to abnormalities in fetal development (through both biological and environmental mechanisms) in a number of studies (Lipner et al., 2019). According to studies (X. Zhang et al., 2004), persons with "schizophrenia" who have elevated levels of the cytokine *interleukin 8* are less likely to benefit from "antipsychotic" treatment. Furthermore, *IL-8* has been implicated in the pathophysiology of the CF disorder because of its function as a signal molecule.

IL-8 is able to bring neutrophils to the lung epithelium and control their movement once they are there. When these recruited neutrophils are overstimulated or don't work right in the airways, they release a number of molecules and proteases that cause inflammation, this, in turn, damages lung tissue even more (Reeves et al., 2011). The adenosine A2B receptor in human mast cells is in charge of making *interleukin-8* come out. Certain benzodiazepines have the ability to inhibit this process. In a study that was conducted in 2013, it was found that diazepam, 4′-chlorodiazepam, and flunitrazepam, in that order of potency, significantly reduced NECA-induced *interleukin-8* production. Clonazepam, on the other hand, showed only modest inhibition (Hoffmannetal., 2013). Through research with preclinical models and clinical trials, it has been shown that blocking *IL-8* with antibodies has positive effects on both non-cancerous inflammatory conditions and cancer (Skov, 2008; Fernando, 2011). Hamilton (2012); Fousek (2021), in a study, the researchers found a direct connection between serum *“IL-8*” levels to disease progression. Multiple ongoing clinical studies show that the way *“CXCL8*” interacts with receptors and “Glycosaminoglycans” could be a good target for therapy.”*CXCL12*”, which keeps leukocytes in the bone marrow, directly counteracts the *CXCL8-mediated* mobilization of neutrophils to the blood. *“CXCL12*”, which keeps white blood cells in the marrow of the bone, directly counteracts “*CXCL8*”-mediated neutrophil mobilization to the blood (Cambier et al., 2023).

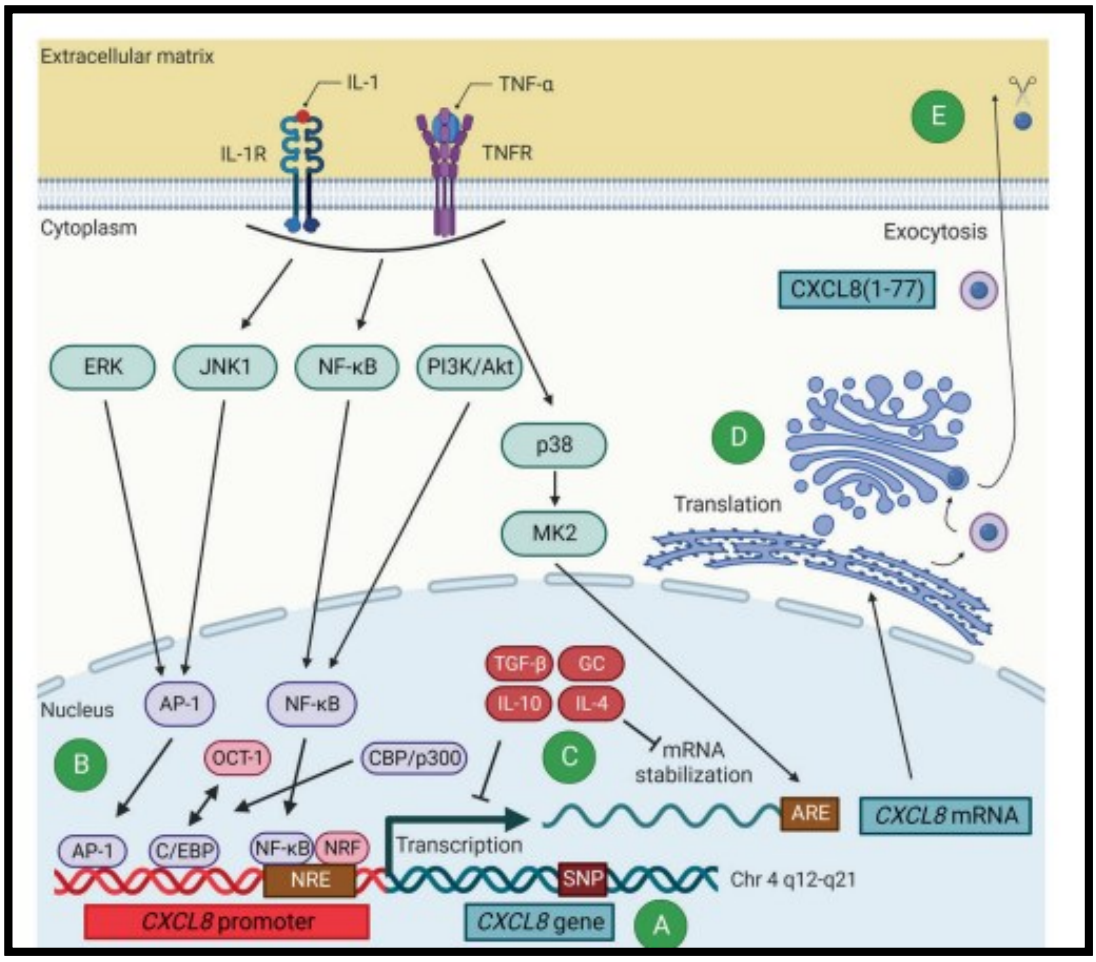
1.2.4. Regulation of expression and Physiological Role of IL 8

It is the case that the regulation of "*CXCL8"* activity occurs on multiple levels, just like the regulation of the creation, release, and activity of other "chemokines" (Cambier, 2023; Li, 2021). This must be done in order to stop the unlimited or improper recruitment of leukocytes, and it may also be essential for the resolution of "inflammation" (Figure 1.1). In response to a local "inflammatory trigger," the

"expression" of the *"CXCL8*" gene elevates after receptor activation by inflammatory agents like *"IL-1*" or TNF-*α*." (Tylek et al., 2021). The next stage, which is the beginning of the transcription process, is the result of a variety of separate signal transduction pathways working in concert with one another. (Fig. 1.1)

Figure 1.1

Inflammatory response mechanism, (Adopted form Tylek et al., 2021)



After inflammatory activation, the synthesis of *CXCL8* is subject to stringent regulation at every stage of the process. Through the activation of their respective receptors, "pro-inflammatory cytokines like *IL-1* and *TNF-α* "are able to stimulate the synthesis of "*CXCL8"* in response to an "inflammatory trigger". This causes downstream signalling pathways to be activated, which leads to the activation of *'NF-kB* and the *AP-1* complex'. These two components then move into the nucleus, where they 'trigger transcription of the *CXCL8* gene' as well as the creation and release of *'CXCL8'*. Several degrees of control are exercised over this process. (A) certain polymorphism of' *CXCL8*' have an effect on the amount of that protein that is produced. (B) Only after the '*CXCL8* gene promoter' has been de-repressed can

transcription begin. This is mediated by the association of *NF-B* with '*NF-B*-repressing factor (NRF) and the negative regulatory element (NRE)' in the promoter. Additionally, the 'octamer-1 (OCT-1)' in the 'promoter' is replaced by the' transcription factor CCAAT/enhancer-binding protein (C/EBP)'. This leads to the' co-activator CREB-binding protein (CBP)/p300 'being recruited, which in turn causes 'histone hyper-acetylation and chromatin remodelling'. This makes it possible for 'AP-1 and NF-B' to activate the process of 'gene transcription'. This transcription mechanism is capable of being halted by anti-inflammatory 'stimuli like IL-10 and TGF-. (C') After synthesis, the unstable "*CXCL8* mRNA" needs to be stabilized by a mechanism that targets "AU-rich cis-elements (ARE)" and is dependent on "MAP kinase-activated protein kinase 2 (MK2)". This is accomplished by activating the "p38 MAPK pathway". This stability is stimulated by "LPS*, IL-1, TNF-α, IFN-γ*, nitric oxide, and hypoxia" while it is suppressed by *"IL-4, IL-10*, and glucocorticoids (GC)", which stimulate the degradation of "mRNA". (D) Following the translation of" mRNA, CXCL8" will localize intracellularly in the "Golgi apparatus". It is from this location that it will be released via the constitutive secretory pathway. (E)After "CXCL8" has been "exocytose", it is susceptible to a wide variety of post-translational changes, including proteolysis, which can have a significant impact on its activity.

There are a few different things that, when they all work together, stop IL-8 from being made. The expression of *"IL-8"* is indirectly repressed by miRNA-146a/b 5p, which does this by stopping the excretion of "IRAK1" (Bhaumik et al., 2009). In cystic fibrosis, interleukin eight and other cytokines that inflammation are responsible for the formation of a virtuous circle with the "transcription factor NF-B" (Rottner et al., 2009). The regulation of NF-kB is an innovative anti-*IL-8* therapy that can be used in the treatment of inflammatory diseases like cystic fibrosis. It has been discovered that the same molecular processes that increase interleukin eight protein synthesis by inducing "ribosomal protein S6 (rpS6) phosphorylation" also increase "rpS6" phosphorylation. Essential for intermediate control of IL-8 secretion are the A/U-rich proximal sequences (APS) found in the "3'-untranslated region (UTR)" of interleukin eight shortly after the stop codon (Ang et al., 2019). Overexpression of IL-8 is found in several different types of cancer, this encourages the development of "mesenchymal" characteristics, cancer stem cells, treatment resistance, and immune-suppressing cell migration to the tumor site. Additionally, *IL-8* is frequently overexpressed in a variety of human carcinomas, such as those of the breast, colon,

cervical, gastric, lung, and ovary (Freund et al., 2003). Both chemical and environmental stresses, such as chemotherapies and hypoxia, as well as inflammatory signals, are recognized to control production of "*IL-8"* (Bilusic et al., 2019). According to Skov 2008; Ransohoff (2005), "IL-8" is in charge for mediating the activation and chemotaxis of immune cells, which ultimately leads to chronic inflammation. According to Perry et al. (2008), changes in the production of microRNAs (miRNAs) in the alveolar epithelial cells of human lung can also affect acute inflammatory responses. *"IL-8"* expression can be inhibited in a number of different ways. Cells use a tiny portion of the "genes" that are present in each cell at any given time to produce "messenger RNA (mRNA)". Genes provide the instructions for producing mRNA. A gene is said to be "off" if it does not contribute to the synthesis of mRNA, and "on" otherwise (Sun, Xi-Ming, et al. 2020). A number of different variables, including the time of day, whether or not the cell is actively dividing, its immediate environment, and chemical signals from cells nearby, can affect a gene's activity. For example, skin cells, liver cells, and nerve cells all activate (express) slightly different genes from one another when compared to one another (Picard, M.,& Shirihai,O.S. 2022). In many experiments meant to profile expression, the relative amounts of messenger RNA (mRNA) made under different experimental conditions are compared, this is due to the fact that modification in the levels of a specific sequence of messenger "RNA" imply a changed requirement for the protein that the mRNA codes for. This can be a sign of a pathological condition or a physiological reaction (Buricet al., 2020). For instance, larger amounts of mRNA coding for alcohol "dehydrogenase" signal that the cells being studied have responded to increased levels of ethanol in their surroundings tissues (Choi, Mi Ran, et al. 2022). In the same manner, if a certain transmembrane receptor is expressed at greater concentrations in "breast cancer" cells than in healthy cells, this suggests that the receptor may contribute to the development of "breast cancer". Normal cells express lower levels of this mRNA. (Liu et al., 2020). During the process of developing a new medicine, it is possible to evaluate the potential toxicity of the drug by employing gene "expression" profiling assays. These tests could involve monitoring changes in the expression of "cytochrome P450 genes", which could act as a biomarker of drug metabolism. (Song et.al. 2021).

1.2.5. Therapeutic target and cells signalling of IL 8

The vast majority of clinical research has been focused on finding ways to block the interaction that occurs between "*chemokines"* and their receptors. This has been accomplished mostly through the utilization of small compounds or neutralizing antibodies that target either the receptors or *the "chemokines*" themselves. However, the results of implementing this technique have only been satisfactory to a limited extent so far. The research was conducted in two distinct settings: in vitro on patient samples and human cells, and in live animals, on mice with a gene defect. Both settings were used to collect and analyze data.

Recent research has shown that *interleukin-8 (IL-8)* can promote the invasion of cancer stem cells (CSC), as well as "metastases" and therapy resistance. Attaching the "CXCR1/2 signaling" "Pathway has been demonstrated to be efficacious in primary aggressive and metastatic breast tumors as well as in in vivo models of breast cancer." As a result, clinical trials evaluating CXCR1/2 inhibitors have begun. "IL-8" primarily targets "neutrophil granulocytes, However, an extensive number of other cells, such as "keratinocytes", endothelial cells, and "macrophages," are affected by it as well and can also react to this chemokine. Keratinocytes are the cells that produce keratin. "Tetrahymena pyriformis" was shown to have chemoattractant activity similar to that of vertebrates when *IL-8* was present in concentrations comparable to those found in vertebrates. This indicates that the structure and function of this chemokine have been phylogenetically well preserved (Kohidai & Csaba, 1998).

1.3. Interleukin 18 (IL-18, interferon-gamma inducing factor)

Interleukin-eighteen gene in humans is in charge of producing the interleukin-eighteen protein, also known as "interferon-gamma" inducing factor (Nolan et al., 1998) The product of this gene, which is a protein, is a cytokine that promotes inflammation (L. Zhang et al., 2019). Many different cell types, including "hematopoietic" and "non-hematopoietic" cells, have the potential to produce interleukin- eighteen (Makaremi et al., 2022). It was not until 1989 that it was discovered to be a variable that triggers the production of "interferon- (IFN-γ)" in mouse spleen cells (Tanaka et al., 2021). At first, it was thought that Kupffer cells, which are resident macrophages in the liver, were the cells responsible for producing "IL-18". Although "hematopoietic cells" do not constitutively produce *"IL-18,"* other non-hematopoietic cell types such as intestinal epithelial cells, endothelial cells, and

“keratinocytes” do. Interleukin-eighteen has the ability to influence both the innate and adaptive immune systems, and a malfunction with its regulation can result to autoimmune or inflammatory illnesses, claim (Beenen 2022; Hachim, 2020). The human heart contains vascular endothelial cells and macrophages, both of which express the *cytokine IL-18*, in their study found that HF patients had elevated serum *IL-18* concentrations, which were linked to lower cardiac functional class and higher TNF-α levels (Miyazaki, 2022; Stanciu, 2019). *IL-18* receptor gene might block the effects of *IL-18*, which would reduce human cardiac reperfusion injury (Stanciu, 2019). *IL-18* is likely a factor in the pathogenesis of HF.

“IFN-γ”, is a “cytokine” that is crucial for “natural and acquired immunity” towards illness brought on by viruses, some varieties of bacteria, and protozoa. IFN-γ has a critical function in both the activation of macrophages and the induction of the production of molecules from the class II of the major “histo-compatibility complex”. Many autoimmune and inflammatory illnesses have been connected to IFN-γ expression. Although its ability to directly suppress the replication of viruses also function in IFN-γ relevance in the immune system, its impacts on immunostimulatory and immunomodulatory pathways account for the majority of this significance. “IFN-γ” is mainly secreted by "natural killer cells"(Lee & Ashkar, 2018; Schoenborn & Wilson, 2007; Wilson & Schoenborn, 2018). In addition to "T cells," "B cells can also produce type II IFN," and "it is feasible for "antigen-presenting cells (APCs)" such as "B cells, macrophages, and dendritic cells" to do so.”Non-cytotoxic innate lymphoid cells" are a class of immune cells that were initially discovered in the early 2010 and are also capable of producing IFN (A. J. Lee & Ashkar, 2018). The synthesis of “type I interferons” as well as interleukin 12, 15 and 18 increases the secretion of “type II IFN” (Artis & Spit., 2015). Because “type II IFN” is a cytokine, it functions by communicating with other immune system cells and regulating how they respond to an infection. “Type II IFN” can have an impact on a range of immune cells. Some of its main actions include “upregulating the expression” of the “MHC class II receptor on antigen-presenting cells (APCs)”, stimulating the differentiation, activation, and expression of “CD8+ cytotoxic T lymphocytes, activating macrophages, inducing B cells to flip their IgG isotypes", and more. “Type II IFN” promotes the “expression” of “IL-12 in macrophages”. Moreover, it delivers a message to “naive T helper cells” “(Th0)”, telling them to differentiate into “Th1 cells”. In turn, “IL-12 increases the production of IFN- γ by NK cells” as well as “Th1 cells” (Artis and Spit2015). Only

“exons 1 and 2 of the IL18 gene's 7 exons” contain any functional genetic material. An early investigation revealed that the mouse “IL18” gene's promoter activity has been discovered “upstream of exons 1 and 2” (Tone et al., 1997).

Research over the past 20 years has revealed that “IL-18” is far more versatile than only an IFN inducer. Many human disorders, including “acute myocardial infarction” (AMI), “heart failure” (HF), and pressure overload, have been associated to an elevation in interleukin eighteen activity. Given the part interleukin- eighteen plays in initiating severe heart effects like muscular malfunction and -AR desensitization, it is conceivable that interleukin-eighteen inhibition could be a helpful technique in both acute and chronic cardiovascular disorders. By genetic deletion of interleukin-eighteen or its neutralization, myocyte hypertrophy can be decreased (Vasques-Nóvoa et al., 2018).

Improving contractile function and reducing infarct size in patients who have suffered an ischemia-reperfusion injury can be accomplished by inhibiting “IL-18” activity through the use of a “neutralizing antibody, *IL-18BPa, NLRP3* inhibitors”, or “caspase-1 inhibitors”. This paves the way for additional research into the role of these molecules in AMI (Aydin et al., 2019). Inhibiting *IL-18*, on the other hand, may have the unintended consequence of impairing the natural “hypertrophic response” to pressure stress and leading to maladaptive remodelling (Lv et al., 2022). There have been no preclinical studies using *“IL-18”*-targeted treatment administered either during ischemia or after reperfusion using *“IL-18BP*” or *“IL-18Ab*”. “Pilot clinical trials in ST-segment elevation AMI (STEMI) and non-STEMI” may be necessary if these investigations demonstrate that IL-18 has a protective effect that lasts over time and is not connected to impaired infarct repair in preclinical AMI modes. *IL-1* blockers have been used in the past to accomplish this O'Brien et.al, (2014). A contrast between *“interleukins” 18* and *interleukin 1* blockers in “acute myocardial infarction” may shed more light on whether the effects of the two cytokines are distinct or cooperative, or whether, interleukin- eighteen facilitates interleukin- one” effects. This may be the case if one of the *“cytokines, IL-18*”, mediates the effects of the other “cytokine, IL-1” (Detry et al., 2022).

Given that *“IL-18*” has a potent cardio depressant effect, inhibiting its activity may prove to be an effective treatment for acutely decompensated or persistently symptomatic heart failure (HF) (Wang et al., 2022). It would appear that *IL-18* influences both the systolic and diastolic functions of the heart (Xing et al., 2010).

Preliminary studies with “interleukin” one blockage suggest that *“interleukin- one”* activity may be adjustable in HF patients” (Van Tassell et al., 2015), and the molecular linkages between “interleukin-eighteen” and “interleukin-one” suggest that blocking interleukin- eighteen may have the identical positive benefits. Both of these lines of research were published in 2015 (Cavalli et al., 2021). Therefore, in people whose levels of IL-1 are high, blocking *IL-18* may be used to improve LV function without having an impact on the other effects that are mediated by IL-Several studies that have been published in academic journals suggest that vascular diseases may be linked to the *interleukin 8 and interleukin18* pathways. To provide information on the expression profiling of *“interleukin 8 and interleukin18*” in veins and the aorta, however, more study is required. This study may reveal further details about these cytokines, notably in veins and the aorta. The synthesis of “interleukins” is absolutely necessary for the development of new blood vessels, which is a process that is speed up by a wide variety of “growth factors as well as cytokines” that can either act directly or indirectly. Interleukins play an essential role in this development (Kang & Kishimoto, 2021). “Macrophages” are primarily responsible for the production of *“IL-18”*, which can also be made by “hepatocytes” and “keratinocytes”. Its primary objective is to inhibit a co-factor that is necessary for the induction of Th1 cells. It does this by causing the production of interferon gamma and by enhancing the activity of NK cells (Vaillant, 2022). Findings of Satş et al. (2021), the concentrations of *“IL-18”* are linked to indices of kidney, liver, and heart damage, as well as indices of blood clotting. , which suggests that it may contribute to pathologic multi-organ injury. According to the findings of a study that was carried out by Bouabdallah et al. (2019). *IL-8* is the most powerful independent predictor of both cardiovascular and all-cause events. The components of the extracellular matrix are responsible for determining the tissue's mechanical properties, striking a balance between rigidity and elasticity. This occurs in all blood vessels (Andreeva et al., 2022). Therefore, alterations and Variations in the rigidity and flexibility of vein walls may be caused by modifications to the “expression” of components of the extracellular matrix as well as their production. These changes may help primary varicose veins develop venous insufficiency. Varicose veins are characterized by the presence of enlarged veins that protrude from the surface of the body. It has been demonstrated that inflammation is a significant contributor to the growth of deep vein thrombosis in the lower extremities, according to some studies and previous research (Pai et al., 2021).

1.3.1. Function of IL-18

The majority of macrophages and a few other cellular components produce *"IL-18",* an *"IL-1* superfamily member". It can perform "pleiotropic" actions and activate a diverse array of cell types. *"interleukin-eighteen"* is a "cytokine" that produces type-one reactions and increases inflammation (Konjevic et al., 2019). It collaborates with *"IL-12"* to trigger cell-mediated resistance in the wake of an infection brought on by microbial by-products like "lipopolysaccharide (LPS)." "CD4, CD8, T cells, and natural killer cells produce "IFN"-γ when "interleukin"-eighteen and twelve are combined. "IFN"-γ, a type II "interferon", is essential for the activation of "macrophages" and varieties of cells (Gocher et al., 2021). The fact that The fact that interleukin-eighteen, in addition to its basic function, is able to cause large inflammatory reactions raises the prospect that it could contribute to the progression of specific inflammatory illnesses, such as persistent inflammatory conditions and auto-immune diseases (Sedimbi et al., 2013). The "neuro-inflammatory response" that develops in the aftermath of an intracerebral hemorrhage can be partially attributed to interleukin-eighteen involvement as well (Gan et.al. 2021).

The *"Interleukin-eighteen* gene's SNP IL18 rs360719 was discovered as an essential variable in the transcription of the "IL18 gene" and to potentially have a role in predicting predisposition to systemic "lupus erythematosus" (Sánchez et al., 2009). While *"IL-1"* has undergone extensive research, *"Interleukin- eighteen* and *interleukin* family have received limited attention, *"interleukin-eighteen"* takes part in the process of regulating the "Th1 reaction by controlling the quantity of IFN-γ that is generated". In this regard, when combined with "Interleukin- twelve or Fifteen, both are known to promote the development of the "IL-18R co-receptor, "IL-18can drive T cells" to produce "IFN-γ (Harel et al., 2022). "IFN-γ is produced by "Natural killer" cells in response *to IL-18,* and "Natural Killer cells" are known to produce large amounts of IFN-γ and to express CCR7" (Barr et al., 2007). In a preclinical "osteosarcoma mouse model". Kaneko et al. (2019) demonstrated that inhibiting "*interleukin-eighteen with IL-18BP* led to a substantial drop in the overall figure of MDSCs in the tumor microenvironment. *IL-18BP* (Tadekinig®) was effective in treating "Still's illness" and "NLRC4-mutated auto-inflammatory Macrophage Activation Syndrome" (MAS), despite the fact that anti-*IL-1* therapy had been ineffective in treating these disorders (Kaneko et al., 2019).

1.3.2. The signalling pathway and Receptor of IL 18

"Interleukin-18 receptor" is made up of two different components: the inducible component *IL-18R*, which has a weak affinity for the mature form of *"IL-18",* and persistently produce co-receptor *IL-18R* (Ge et al., 2019). When *"interleukin-eighteen binds*" to the "ligand receptor *IL-18R",* it induces the activation of *"IL-18R"* to form a "high affinity complex". After that, this complex communicates by way of the *"toll/interleukin-1* receptor (TIR) domain" (Fields et al., 2019). This signaling domain is responsible for recruiting the MyD88 adaptor protein, which is what activates the pro-inflammatory programs and the *NF-B* pathway. *Interleukin-eighteen* binding protein *"IL-18BP"* is an "extracellular" protein that "binds soluble *IL-18* with a higher affinity than *IL-18R* and thus prevents *"IL-18"* from attaching to *"IL-18 receptor."* This protein is responsible for reducing the levels of *"IL-18"* in the body (Dinarello et.al., 1999). An additional internal component that prevents the activity of *"IL-18* is *IL-37",* which plays this role. High levels of homology exist between *"IL-37* and IL-18" is able to bind to *"IL-18R",*which then forms a complex with *IL-18.*Signaling that is mediated by *"IL-18"* is responsible for the effects it has by sending signals through its receptor, which is a member of the *IL-1R* family and is made up of two chains: the *"IL-18R* chain *"(IL-18R1, IL-1Rrp)"* (Ferraz et al., 2022). *"IL-18R"* will proceed to complete its own binding to produce a trimer after IL-18 has completed its binding to *IL-18R*, "MyD88 binds" to "TIR", which allows it to send a signal into the cell. This is because TIR and the intra-cellular region both have a similar "TIR domains". Although *IL-18R* "can bind to IL-18" on its own, the attraction between the two molecules is relatively weak (Yasuda et al., 2019). It is necessary for potent IFN-γ production to have *IL-18R* expression, which can be induced in "T cells and NK cells" by stimulation with *"IL-12* and IFN-γ (human) or by signal transduction and transcriptional regulation via STAT4" (Signal transducer and activator of transcription 4) (Khalil et al., 2021). Epithelial and nerve cells are examples of non-immune cells that are able to express *IL-18R*, and this protein has part in both survival and variation of cells in the body. There is a lack of clarity regarding the regulatory mechanism that controls the manifestation of *"IL-18R*" in these cells.

1.3.4. Clinical significance and metabolic homeostasis of IL 18

In addition to its function in the body's physiological processes, the cytokine known as *interleukin-18 (IL-18)* has been shown to be capable of inducing severe inflammatory reactions, which points to a potential role for it in some inflammatory diseases such as chronic inflammation and autoimmune illnesses (Dinarello, 1999). Hashimoto's thyroiditis, a prevalent “autoimmune” disorder that results in “hypothyroidism”, may involve the participation of *"IL-18"* as an inflammatory mediator (Zhang, 2022; Karakaya, 2021). The present study has revealed that the expression of amyloid beta, which is associated with “Alzheimer's” disease, was augmented in human neuron cells due to the influence of *“IL-18”* according to research published by (D'Ascola 2020; Ennerfelt, 2020, Liu , 2010). The potential of *"IL-18"* to function as a marker for determining the progression of diabetic nephropathy can be attributed to its association with urine protein release (Fengxun et al., 2015). In comparison to individuals who are in good health and those with diabetes who exhibit normal levels of “albuminuria”, patients with microalbuminuria and macro-albuminuria demonstrate notably heightened levels of this particular interleukin (D. Zhang et al., 2019). The cytokine known as *"IL-18"* plays a role in the “neuro-inflammatory” response that occurs following “intra-cerebral bleeding” (H. Zhu et al., 2019). Interferon-gamma is responsible for the increased production of *interleukin-18* (Avagimyan et. al., 2021).

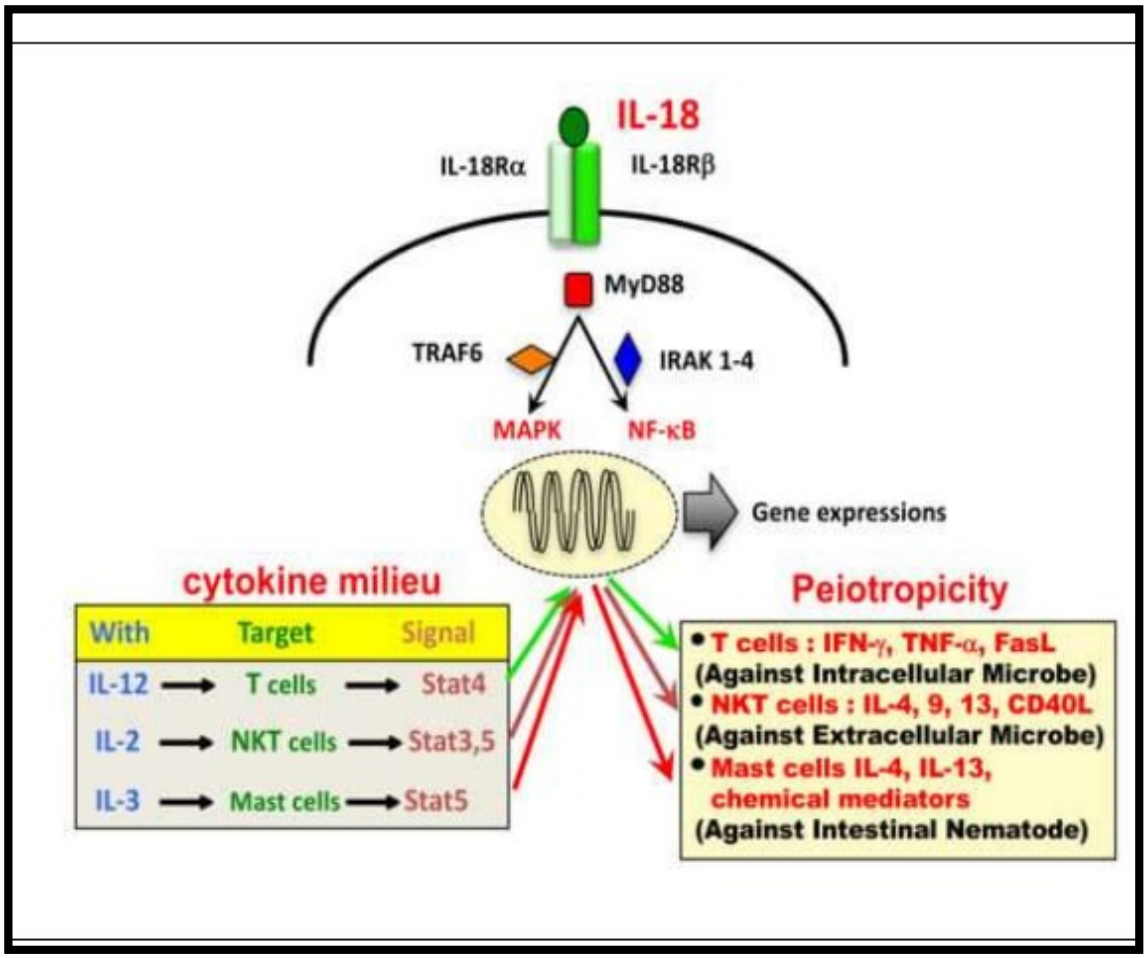
Inflammatory bowel conditions, autoimmune disorders, cardiovascular function, lung disease, syndromes of metabolism, psoriasis, hemophagocytic syndromes, stimulation of macrophages syndrome, sepsis, and acute kidney injury have all been associated with *IL-18”,* but in other disease models, it is protective (Ali et al., 2022).

Ihim et al., (2022) in their work revealed that both “hematopoietic” and “non-hematopoietic cells”, such as “monocytes”, “macrophages”, “keratinocytes”, and “mesenchymal cells”, produce *"IL-18."* Additionally, when “LPS binds to TLR4”, “caspase-1” is activated, leading to the production of *“IL-18”.* Numerous additional cells, such as “keratinocytes, intestinal “epithelial cells”, and “osteoblasts”, also create “pro-*IL-18”,* showing that it plays a large pathophysiological function in both health and sickness in addition to these *“IL-18*-producing cells”. Depending on its “cytokine milieu”, *“IL-18* exhibits pleiotropic” activity like other “cytokines” do. Figure 1.2 illustrates that the pleiotropic action of “IL-18 depends on its “cytokine milieu”.

Recent research has demonstrated that administering “IL-18” “intracerebral” ventricular extends non-rapid eye movement sleep, which points to a role for “IL-18” in the regulation of sleep.

Figure 1.2.

Pleiotropic action of IL-18 depends on its cytokine milieu. (Adopted from Ihim et al., *(2022)*



1.3.4. Regulation of expression and Physiological Role of IL 18

In addition to having an impact on the immune system, *"IL-18"* also affects other physiological functions and systems, including the neurological system, endocrine system, and bone metabolism. Since the adrenal gland makes *IL-18* when it is stressed, this support the fact that *IL-18* could be involved in the changes that stress makes to the body as a whole. The fact that *IL-18* produced by osteoblasts suppresses osteoclast differentiation while simultaneously promoting GM-CSF synthesis highlights the crucial role of *IL-18* in regulating bone metabolism. "IL-18" is said to be kept in the "cytosol of *IL-18*-producing cells" (González et al., 2022).

Consequently, the "extracellular release of biologically active *IL-18"* is controlled in several ways, including the more common "transcriptional gene regulation," "post-transcriptional gene regulation," and" post-translational gene regulation"(Yasuda et al., 2019). Interleukin-18 (IL-18) is a multifaceted cytokine that plays a role in both acquired immunity and innate immunity. Its responsibilities encompass both of these types of immunity. It has been hypothesized that its principal function is to stimulate the generation of interferon-gamma (IFN-γ) by either T or NK cells due to its high capacity for doing so (Okamuraetal., 1998) . *IL-18* can stimulate the synthesis of IFN-γ in "T, B, and NK cells", according to research by Markova, (2022); Mace, (2019.This effect is exacerbated in the presence of *"IL-12".* In addition, *"IL-18"* is a co-factor that boosts the production of "IFN-γ, *"IL-2",* GM-CSF, and "IL-2R" in Th1 cells" but has no effect on "Th2 cells" (Ashrafzadeh-Kian et al., 2021). This is because *"IL-18"* Th1 cells, according to L. Chen et al. (2022), *"IL-18R"* is solely expressed on Th1 cells". As previously noted, IL-18 is an extremely powerful cytokine that possesses the capacity to impact not only the immune system but also non-immune systems, including the "osteoarticular" and "endocrine systems" (Ihim, 2022; Nakanishi, 2001).This gives us the ability to presume that it must play many roles in the pathogenic processes that are taking place, as stated by (Alboni et al. 2010).

1.3.5. Therapeutic target and cells signalling of IL 18

Yasin et al. 2020) determined that *"rIL-18BP*" in individuals with "refractory sJIA was well tolerated with reduced MAS" frequency and intensity. In addition to the dramatic improvement in linear growth shown after weaning from steroids, they also discovered that *"IL-18BP*" was linked to the stability of the sickness trend and reduced the degree of "MAS." Despite persistently elevated total serum *"IL-18"* levels, "free *IL-18"* levels became undetectable shortly after therapy with *"rIL-18BP"* was begun. Consistent with previous research, this data suggests that "free *IL-18"* may be a more accurate biomarker for the disease's progression than previously thought.

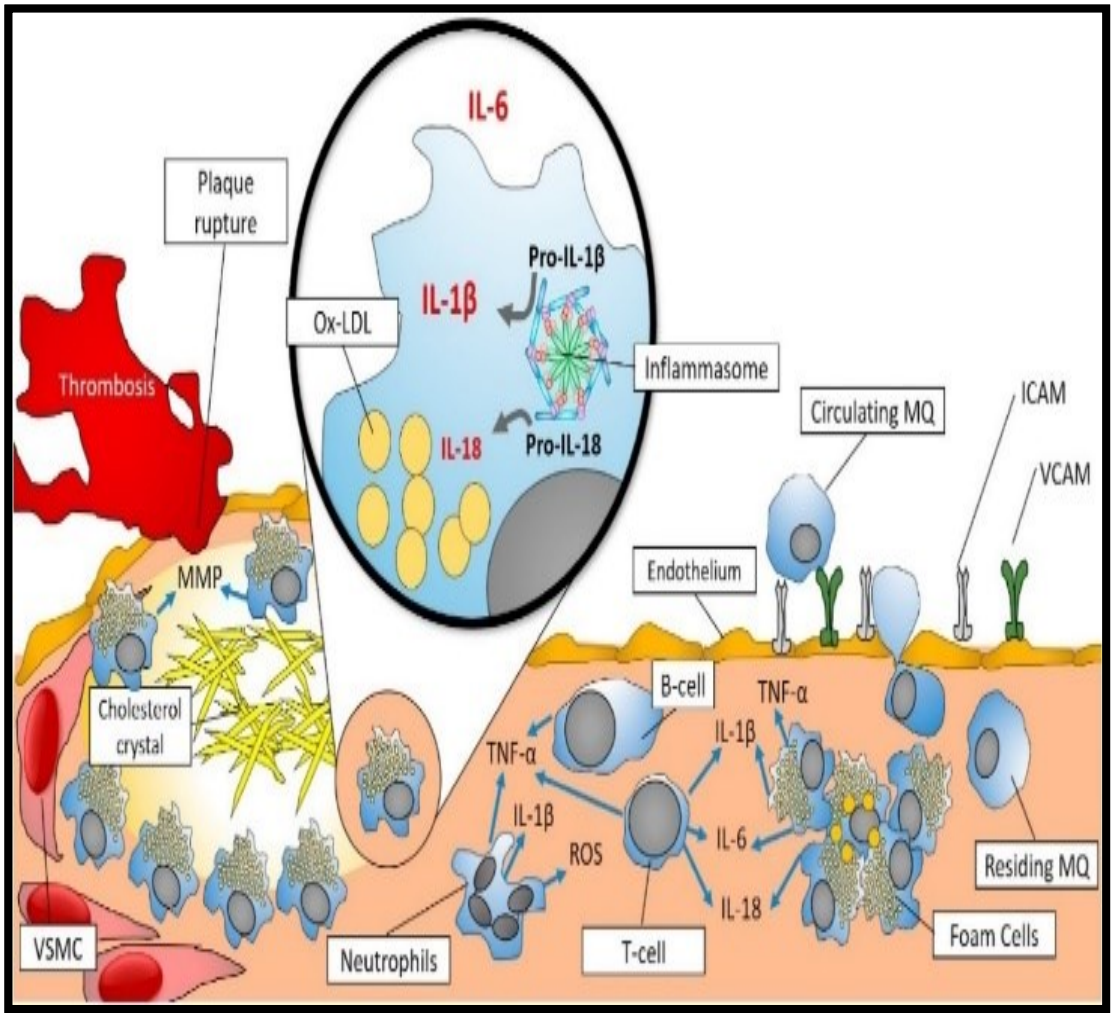
1.4. Inflammation and CVDs

Inflammation has long been recognized as one of the main causes of many diseases. According to Piotrowski et al. (2020) 20% of all human malignancies are thought to be related to chronic inflammation and infections. The heart, pancreas, liver, kidney, lung, brain, gastrointestinal tract, and reproductive systems" are just some of the

organs where inflammation has been shown to cause both short-term and long-term tissue damage (Kaur & Singh, 2022). Atherothrombosis is a multi-step process, one of the most important drivers of which is inflammation (Alfaddagh et al., 2020g). (Fig. 1.3). The sub intimal inflammatory response is what makes atherosclerotic lesions stand out. It begins with a buildup of cholesterol in the sub intimal space and impaired endothelial function. Overexpression of attachment molecules such intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and others allows inflammatory cells like monocytes and T helper cells to attach and roll to initial lesion formation regions. Monocytes that invade the sub endothelial area have the capacity to develop into local macrophages (Liang et al., 2020). A critical step in the progression of inflammation is the activation of the inflammasome in macrophages (Soehnlein & Libby, 2021). When macrophages that have been primed by the nuclear factor kappa B (NF-kB) pathway encounter a second stimulus, such as cellular hypoxia or ingested cholesterol crystals, the inflammasome, a complex cytosolic multiprotein, is formed. Multiple proteins work together to form the inflammasome. Inflammasome maturation leads to IL-1 production from pro-IL-1 (Jin & Fu, 2019). Similarly, *pro*-IL-18 is cleaved to IL-18, the functional version of the molecule. Many inflammatory cells are triggered by the secretion of these cytokines, leading to the production of IL-6. Therefore, the inflammatory process within the arterial wall is amplified, as the liver is prompted to create CRP (Hareletal, (2022).

Figure1.3.

Inflammatory response cascade (Adopted from Alfaddagh et al., 2020)



Inflammation is a central factor in each stage of the atherosclerosis process, as shown in the figure legend. *ICAM, VCAM, IL, MQ, MMP, VSMC, and Ox-LDL are acronyms for intercellular adhesion molecule, vascular cell adhesion molecule, interleukin, and metalloproteinase*. *VSMC* stands for *vascular smooth muscle cell*. *Ox-LDL* is an acronym for oxidized low-density lipoprotein. The development and spread of “atherosclerotic plaques” are significantly more difficult due to the layered and redundant mechanisms in the “inflammatory cascade”. As a direct consequence of this, an increased amount of *"cytokines*," *"interleukins*," and “reactive oxygen compounds” that consist of “peroxide”, “superoxide anion”, and “peroxy-nitrite” are created (Alfaddagh et al., 2020). Other inflammatory cells, such as “T cells”, mast cells, and “dendritic cells”, are also implicated in the creation and development of plaques. These cells contribute to the enhancement of "cytokine" production and signalling (for example, by releasing a large amount of "interferon and the tumor necrosis factor"

(TNF) (Ren et al., 2023). An "erotic lipid core" forms when lipid-rich macrophages, also known as foam cells, accumulate due to faulty "macrophage efferocytosis" (Orekhovetal., 2020). By controlling collagen synthesis and destabilization in the fibrous cap, inflammation also contributes significantly to the continued viability of complex "atherosclerotic plaques". The presence of inflammation also plays a major role in influencing the likelihood of atherosclerosis development under these conditions. As a result of cytokines released by foam cells, T-cells, and other cells, vascular smooth muscle cells move into the intima and form interstitial collagen. The extracellular matrix around the necrotic lipid core is composed of these elements working together. For example, (A. L. Khan et al., 2021) matrix "metalloproteases" 1, 8, and 13 are responsible for collagen breakdown in the fibrous cap.

Acute coronary syndromes and myocardial ischemia are caused by the thinning of the fibrous cap, which increases the risk of rupture and the formation of an overlying thrombus. Matrix metalloproteases 1, 8, and 13 are produced in part by *IL-1* along with the growth of the lipid core (Lubrano & Balzan, 2021). Extreme reduction of “low-density lipoprotein cholesterol” (LDL-C) with statin medication can significantly reduce cardiovascular events (CVD) (Cho et al., 2019). Our view of atherosclerotic cardiovascular disease (ASCVD) has changed from one in which chronic inflammation is the primary cause to one in which passive cholesterol accumulation is the cause. Atherosclerotic plaque forms and rupture events are triggered by a cascade of biochemical and histologic mechanisms triggered by chronic inflammation. Atherosclerotic cardiovascular disease (ASCVD) was previously thought to be a condition caused by passive cholesterol build-up (Alfaddagh et al., 202l).

1.5. Cardiovascular diseases: deaths, death rates and premature mortality

Among the leading causes of death in developed countries is heart illness, and atherosclerosis's clinical impact is even more pronounced than it already is (GAO, Zujie, et al. 2019). There are a number of other factors that can cause damage to the arterial wall and contribute to the manifestation of the disease. One of the key contributing elements to the onset of atherosclerosis is chronic inflammation (Li et.al. 2020) Research (Fatyga, Paulina, et al., 2020). shows that *interleukin-8* is a key factor in figuring out how likely it is that a person will die from heart disease.*IL-18* has been proven to be a predictive marker in patients with severe sepsis and septic shock, in addition to being a robust predictor of heart events in older men with metabolic

syndrome (Trseid, Marius, et al. 2009). This was discovered through a number of studies that were conducted (Korpelainenet al. 2020). In diabetic patients, having an elevated level of “IL-18” can be a factor that contributes to nephropathy and atherosclerosis progression (Hirooka, Y., & Nozaki, Y., 2021). Atherosclerosis is an inflammatory disease, and signs of inflammation are also major predictors of death, which has become clearer over the past few years (Keeter, W. Coles, et al., 2022).

Interleukins are proteins that are important for the stimulation and differentiation of immune cells, as well as for the growth, maturation, migration, and sticking together of immune cells they also have both properties that make inflammation worse and properties that make it better (Zhu, 2021;Scherger, 2020). So, controlling development, separation, and stimulation is the primary function of interleukins in immunological and inflammatory responses (Masoomikarimi et al.; 2022). Heart disease, primarily atherosclerosis, is the largest global cause of mortality and disability, which is also the disease's underlying pathology (X. Jiang et al., 2023). According to Gandhi and Raghava's 2023 study, "The EBMT Handbook," 2019 projects that by the year 2030, cardiovascular diseases will be responsible for the deaths of nearly 23.6 million people each year. Inflammatory mediators are very important in atherosclerosis, from the time that leukocytes are recruited until the plaque breaks (Huilcaman et al., 2022). Inflammation is also a very early sign of cardiac stress. Affected cardiac tissues have higher quantities of endothelial attachment molecules and make and release more inflammatory cytokines and chemokines (Huilcaman, 2022; Zhazykbayeva, 2020). The natural immune system is the first line of protection for the heart against pathogenic organisms and harm to cardiac tissue (Lafuse et al., 2020). The most common cause of heart damage is myocardial infarction, which typically occurs as a consequence of coronary atherosclerosis and is characterized by the sudden death of a significant number of myocardial cells (Michaud et al., 2020). Cell death results in the discharge of intracellular components that activate innate immune mechanisms, which in turn initiate an inflammatory response (Ma, 2021). This response is necessary for the removal of dead cells and debris from the infarct. Necrotic cardiac cells are responsible for this process. Endogenous ligands that are released after damage are recognized as danger signals by cell surface receptors, which turn on the inflammatory response (Anderton et al., 2020). People who have diabetes have a higher risk of developing cardiovascular disease, which accounts for approximately two-thirds of all deaths in

this population. According to Barrett-Connor (2018), ischaemic heart disease accounts for about 40% of these fatalities, while other heart diseases, primarily congestive heart failure, account for 15% of deaths. Recent estimates from around the world suggest that more than four hundred and twenty two million people are presently living with, where more than ninety percent have type 2 “diabetes” (Saeedi et al., 2019).

1.6. Vein development and formation

All animals, including humans, have veins in their circulatory systems; these are the channels responsible for transporting blood from the body's extremities back to the heart (Bit & Suri, 2020) the majority of veins are in charge of returning oxygen-poor blood from the body's tissues to the heart, which is their primary function. On the other hand, the veins that are part of the pulmonary circulation and the fetal circulation are the ones that are in charge of carrying oxygenated blood to the heart (J. M. Kim, 2022). There are three various types of veins, which are referred to as large, medium, and small, respectively. The venules that have the lowest diameter are called "post-capillary venules," and they are minuscule veins that make up the veins that make up the tiny blood vessels. “Venules” are veins that are smaller than capillaries. (Murrant & Fletcher,2022; Coccarellia & Nelson,2023).The development of normal veins follows a pattern that is characterized by the gradual emergence of a sequence of paired venous structures, the establishment of anastomotic channels connecting these structures, and the eventual selective regression of particular segments. This pattern describes the path of normal venous development. At first, the systemic venous tributaries are mirror images of one another on both sides, exhibiting perfect symmetry (Subramanyan et al., 2023). On each side, they converge to create a single structure, which subsequently opens into paired channels that are known as the sinus horns and are connected to the growing heart tube. These sinus horns are connected to the developing heart tube (Hikspoorsetal., 2022).

1.6.1. Clinical significance

The vast majority of venous diseases are characterized by blockages such as thrombi or insufficiency of the valves, or both (Krasiski 2021; Alsaigh, 2021). Inflammation and/or compression may also contribute development of other disorders. When it comes to venous illnesses, one of the most significant independent risk factors is one's age (Singh, 2022).Venous insufficiency Spider veins and varicose veins are

both prominent symptoms of “venous insufficiency”, is the most prevalent condition of the “vascular “system (Singh, 2022). Insufficiency of the veins is the most common condition that affects the veins and venous system. Compression therapy, vein stripping, ambulatory phlebectomy, foam sclerotherapy, laser treatment, and endovenous thermal ablation are some of the potential treatments. Endovenous thermal ablation can be conducted using radiofrequency or laser radiation. Deep vein thrombosis, also known as postphlebitic syndrome, is a kind of venous insufficiency that can occur in patients who have previously been affected by the condition known as deep vein thrombosis (DVT) (Patel, 2021; Bootun, 2022; Lohr, 2021).

1.6.2. Thrombosis of the veins

When a blood clot, or thrombus, forms inside of a vein, it causes a medical condition known as venous thrombosis. The most prevalent form of this disorder is called deep vein thrombosis (DVT), but when it affects a superficial vein, it is called superficial vein thrombosis (SVT) (Budnik & Brill, 2018; Menéndezetal., 2016). Although it is more common in the veins of the legs, “deep vein thrombosis (DVT)” can also take place in the “deep veins” of the arms (Broderick et al., 2021). Inactivity, active malignancy, being overweight, psychological damage, and “hereditary” illnesses that enhance the possibility of blood clots are all risk factors for developing deep vein thrombosis (DVT), which is a condition that affects the deep veins in the body. This makes it possible for the affected limb to enlarge, in addition to generating pain and a rash on the skin that covers the affected limb (Cushman, 2007; Khan, 2021). When a portion of a blood clot breaks off as an embolus and becomes lodged in a pulmonary artery in the lungs, this is known as a pulmonary embolism (Zakirovna, 2022; Admin, 2023). A deep vein thrombosis might potentially spread, or an embolus could form if a piece of the clot broke off and travelled through the bloodstream.

The severity of the condition, the symptoms it causes, and the other risk factors all play a role in determining whether or not treatment is necessary. In most cases, anticoagulation is used as a means of preventing blood clots or of reducing the size of existing ones (Sagris, 2022; Duffett, 2022). In patients suffering from edema or those who are at risk of developing deep vein thrombosis, intermittent pneumatic compression is a therapy that can be utilized to improve venous circulation (Al-Dorzi et al., 2022).

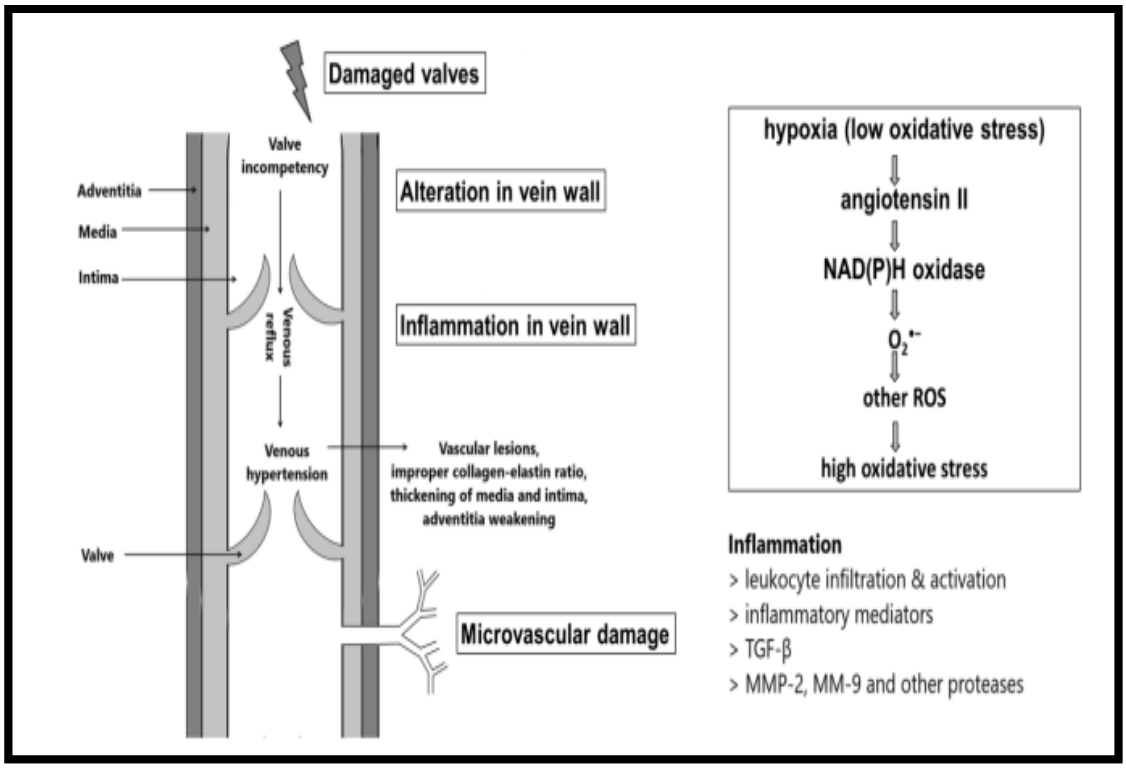
1.6.3. Venous insufficiency

A vein that is twisted and swollen that can be seen just beneath the skin's surface is referred to as a "varicose vein." It usually happens in the legs. Varicose veins can be painful. According to the findings of epidemiological studies, the incidence rate can range anywhere from 2% to 56% in men and 1% to 60% in women (Lichota et al., 2019c). The most prevalent cause of venous insufficiency is damage to the veins' valves and walls (DePopas & Brown, 2018). The development of varicose veins is a complicated process, but it is supported by "hypotensive blood vessels, hypoxia," and other factors connected to inflammation (Lichota et al., 2019). In addition to being more common during pregnancy, risk factors for varicose veins include being overweight, not getting enough exercise, sustaining injuries to the legs, and having a family history of the condition. According to Lohr and Bush (2013), varicose veins are extremely common and will affect approximately thirty percent of people at some point in their lives (Baram et al., 2022). Age tends to exacerbate the problem's prevalence. According to Abou-ElWafa et al. (2020), women are more likely to develop varicose veins as compare to men (Whitley, 2022) .Most people with varicose veins have valves that don't close well, which causes blood to pool in the vein and eventually leads to venous hypertension (Berthelot et al., 2022). It is hypothesized that this disorder is what causes inflammation and the “oxidative stress” that goes along with it. Incompetent valves are associated with varicose veins, this result to venous hypertension, which can cause vascular lesions (an improper collagen-elastin ratio as well as medial and intimal thickening). Varicose veins are unsightly and can be painful (Lichota et al., 2019). The stagnation of blood and compression of the vasa vasorum that result in medial hypoxia are both caused by stretching, which is a secondary cause of increased hydrostatic pressure. It is possible for venous hypertension to spread from the veins to the small blood vessels. This can cause changes in the capillaries, which in turn can lead to edema, skin damage, and ultimately grow venous ulcers. Venous hypertension, damage to the venous valves (regurgitation), and injury to the calf muscle pump can all cause changes in the vasculature that are indicative of chronic venous disease (Blebea, 2023). Venous hypertension, which is the primary cause of CVI as well as valve failure, most frequently causes both conditions. Changes to the capillaries that take place when this illness enters the microcirculation are what lead to edema, skin damage, and other CVI symptoms (Lichota et al., 2019). DVT, commonly referred to as deep vein thrombosis in the lower extremities, is a common

peripheral vascular condition and an important contributing factor to the onset of this illness is inflammation (Pai et al., 2021c).

Figure 1.4

Deep vein thrombosis (Adopted from Lichota et al., 2019)



Damage to the vein eventually leads to the formation of varicose veins (Gawas, 2021; Labropoulos, 2019). Chronic venous illness is a result of vascular hypertension that persists for an extended period of time due to faulty valves, outflow blockage, or calf muscle pump failure. Prolonged venous illness develops as a result of this hypertension. Blood stasis causes hypoxia and long-lasting inflammation, which subsequently in turn alters the vein walls, causes leukocyte adhesion and activation, releases inflammatory mediators and ECM (extracellular matrix extinction enzymes) like “MMP-2” and “MMP-9” “metalloproteinases”, and releases “TGF” (transforming growth factor), which can cause “fibrosis”. When this happens, the neutrophils are also activated, which causes a considerable amount of “superoxide anion radicals” to be released these radicals served as building block to other “reactive oxygen species”. It has been shown that when oxygen levels drop, the oxidoreductase gene becomes more active. Increased ROS production causes oxidative stress, which eventually leads to the development of varicose veins. Venous hypertension changes the way blood flows through capillaries. This makes leukocytes stick together and become active, which

starts an inflammatory response. Utilizing flavonoids reduces inflammation, neutralizes “reactive oxygen species”, and repairs damaged blood vessels. Due to poor blood flow in the affected leg, severe varicosities can have major effects. Some of these effects are pain, stiffness, and being unable to walk or stand for long periods of time.

A medical disease called “chronic venous insufficiency”, or CVI, causes blood to pool in the veins, causing pressure to build up against the vein wall (Azar et al., 2022). The condition is known as chronic venous disease if the impaired vein function causes significant symptoms such as ulcer formation and swelling (Gastaldi et al., 2021). The most common reason for CVI is a condition called superficial venous reflux, which can be treated successfully (Scott et al., 1995).This condition typically affects the lower extremities, because healthy venous valves are necessary for efficient blood return from the lower extremities (Fernández-Colino & Jockenhoevel, 2021).

1.6.4. Etiology

An obstruction in the veins or dysfunctional vein valves can both lead to venous insufficiency. Venous insufficiency is a medical emergency. When this occurs, blood that should be flowing upwards toward the heart instead flows back down into the legs (Adler et al., 2022). Venous reflux is another name for the flow of blood in the opposite direction of what it should be (Youn and Lee (2019). One of the specific variables that may contribute to venous insufficiency is the production of blood clots in the deep veins of the legs. This can be a very painful condition (Shatri, 2023).

Deep vein thrombosis, which is more commonly referred to by its acronym DVT, is the condition that is responsible for venous insufficiency more often than any other medical condition. As a result of the clot's presence in this scenario, the normal flow of blood is obstructed, which leads to an increase in the pressure within the veins. The vein valves, in turn, become stressed and eventually destroyed as a result of the increased pressure, which produces the increased pressure. Sometimes it's just a natural part of getting older, but prolonged periods of standing or sitting might cause the vein valves to become more susceptible to damage. Malformations are present at birth in the vein valves (Nelzén, 2023). What this indicates is that there has been an issue with the valves ever since the patient was born. Due to a lack of physical activity or diminished mobility as a result of a multitude of factors such as aging or an injury, in extremely rare instances, venous insufficiency might be a result of pelvic

malignancies (Whing and Howard, 2022; Strolin and Kofler,2020). There are a few risk factors that, when combined, put a person at a higher risk of having venous insufficiency. If the patient is classified into any of the following categories, the likelihood that they have venous insufficiency is much higher than it would be for an average person: Risk factors include being female, having “May Thurner syndrome”, injuring a vein, standing or sitting for long periods of time, being pregnant, smoking, being over the age of 50, and having a personal history of varicose veins. Inactivity is a contributing element as well (Patel, 2022; Ashrani, 2009).

1.6.5. Molecular pathophysiology and genetic profile

The function of genes that code for inflammatory compounds and other warning signs for heart disorder has been studied genetically across the population; this research has led to important new insights. Wu et al. (2019) found that there are a number of “polymorphisms” in the genes that control how much cytokines and inflammatory markers are in the blood. The “IL-8 gene”, which is located on “chromosome 4q12-q21”, is responsible for the expression of the “IL-8 protein”, which is a very small protein (Elsaie & Aly, 2022). The question of why some inflammatory responses can be different in people who are exposed to the same risk factors may be answered by studying how the genes of inflammatory mediators are expressed in different ways.

Additionally, “Mendelian randomization” studies provide insights into potential inflammatory targets that contribute to cardiovascular disease (CVD) (X. Li et al., 2021). Chromosome 2 contains the genes that code for *interleukin-1 alpha* and *interleukin-1 beta*. There has been a discrepancy in the genetic data associating variants of genes that are engaged in *IL-1* expression (Heidari et al., 2019). The *Interleukin 1* Genetic Consortium found a link between a genetic variant that increased the expression of an *interleukin-one inhibitor* called *“IL-1Ra*” and an increase danger of coronary artery disease development. Mendelian randomization research discovered this association (Oikonomou et al., 2022).

In an integrated meta-analysis of eighty-two papers, it was discovered that small mutations at rs2228145 and rs7529229 were associated with a decreased incidence of “coronary artery disease (CAD)” without affecting blood lipid levels, blood pressure, or body fat. In the studies, IL-6R polymorphisms were evaluated at sites that were known to have an impact (Alfaddagh et al., 2020f).

1.6.6. Interleukins and venous insufficiency

It is estimated that forty percent of adults suffer from the ailment known as “venous insufficiency”, which is fairly common (Kolluri et al., 2020). As you get older, there is a greater possibility that you will develop “venous insufficiency” (Azhdari et al., 2020) in advanced cases of "venous insufficiency," the skin on the patient's lower legs may develop ulcers or open sores, this is possible if the patient suffers from venous insufficiency. These ulcers are referred to as “venous stasis ulcers” in the research that was conducted in Raffetto et. al. (2020). These ulcers come about when the blood pressure and swelling in the affected area grow to the point where the capillaries in your body, which are very small blood vessels, either burst or become damaged. This causes blood to seep out into the surrounding tissue, which leads to the development of these ulcers (Schneider, 2021; Kumar, 2022). The damaged area of skin will become more sensitive to the effects of any injury once this happens, and it will develop yellow and red patches beneath the skin that are visible. Ulcers aren't the only thing that can result from broken capillaries; sometimes the surrounding tissue can become inflamed as well. Unfortunately, not only is it difficult to repair ulcers brought on by venous stasis, but you also run the danger of acquiring major issues as a direct result of having ulcers in the first place.

The infection that results from these ulcers is the most significant risk associated with them. If the infection is not treated appropriately, it can spread and lead to cellulitis, which is a condition that could result in death (Krzyszczyk, 2018; Panuncialman & Falanga, 2010).

Elevated levels of IFN-y and *IL-18* production are associated with several autoimmune diseases that affect humans, this is according to research by Rackov et al. (2022). It is believed *that IL-18* has a role in the development of systemic lupus erythematosus, rheumatoid arthritis (RA), type 1 diabetes, Crohn's disease, psoriasis, and graft-versus-host disease (Yang, Day, et al., 2021).

There have been many reported successes with anti-*IL-18* therapies. A phase IIa trial using the “anti-*IL-18 monoclonal”* “antibody GSK1070806” to treat “type 2 diabetes” showed that it was safe to use across many sites and in comparison, to a placebo and active treatment. But the use of anti-*IL-18* drugs did not enhance glucose regulation (Kaneko et al., 2019c). Utilizing chromatography on *IL-18* beads, *interleukin-eighteen* binding protein (IL-18BP) was isolated from urine, and it inhibited the induction of interferon beta, *interleukin-8*, and nuclear factor B in vitro

(Kaneko et al., 2019c). Heparin is a naturally occurring glycosaminoglycan that acts as a blood thinner and counteracts the effects of histones, making it useful in the treatment of stable angina and heart attacks (Fuchs et al., 2011). In the majority of CVI patients, the deep vein system or the superficial vein system can be treated with a stent. Varicose veins, for instance, can be treated, among other things, by endovenous surgery performed under local aesthesia.

1.6.7. Treatment

The primary symptom of venous insufficiency is an inadequate flow of blood in the veins, therefore, therapy for this condition focuses on restoring the normal flow of blood through the veins. This is done by ensuring that the blood moves in the appropriate direction (Spaide., 2022; Arbaaz, 2021). The following factors will be factored into the formulation of the treatment plan: age, the severity of the venous insufficiency as well as its prognosis, the symptoms that the patients are reporting, and the drugs that they are currently taking. This is significant because certain medications that could be provided to you might have unfavorable interactions with any other prescriptions you might be taking today, as well as with your overall health and wellbeing (Zentner, 2020; Handelsman, 2021).

1.6.7.1. Medication (Diuretics). Diuretics, which are commonly referred to as water pills, are medications that are taken in order to encourage the kidneys to remove excess fluid from the body (Smith, 2022; Gabriel & Idu, 2021). If the patient have any edema that is connected with "venous insufficiency", these medications could be recommended by the doctor to help in the treatment of it. Antibiotics: In the case that the patient's open ulcers from venous insufficiency get infected, the doctor may prescribe antibiotics. This is due to the increased risk of infection presented by open ulcers. If you have venous insufficiency, you are more likely to develop open ulcers.

Pentoxifylline is a medication that is frequently used in alongside with compression therapy in order to hasten the healing process of venous ulcers (Jull et al., 2012). Anticoagulants, often known as blood-thinning medication, are one type of recommended drug.

"Endovenous laser ablation" is a sort of "minimally invasive" therapy that employs laser heat from a catheter or "cannula" to heat up and shut damaged veins. The catheter, or cannula, is inserted into the damaged veins to administer the laser heat. This

procedure is effective in treating a number of vein problems, including varicose veins and “spider veins”, among others. Once blood is unable to travel via certain veins, there will be less blood pooling in those areas. As a consequence of this, circulation will be enhanced because the blood will be redirected to other veins that are healthy and operate appropriately. In addition to eliminating varicose veins and contributing to the healing of venous ulcers, endovenous laser treatment can eliminate spider veins.

During this treatment, local anaesthesia will be administered (Yao, 2022). “Radiofrequency ablation”: This procedure is extremely comparable to “endovenous laser therapy”; the sole distinction is that radiofrequency energy, rather than lasers, will serve as the source of heat throughout the ablation process (Mese, 2015; Gale , 2010; Redenius & Mann, 2019).

1.6.7.2. *Sclerotherapy.* Is a process in which special chemical solutions are injected directly into the veins that are problematic for human health. After being exposed to these toxins, the veins will get scarred, swollen, and eventually close off. Blood that formerly flowed through them will be diverted to other healthy veins, resulting in the disappearance of visible varicose veins or at the very least a significant fading of their appearance (Mayo Clinic, 2022).

1.6. 7. 3. ***Surgery.*** There is a variety of surgical treatment options available, including ligation. During this particular surgical operation, the afflicted veins will be tied off so that blood will no longer be able to flow through them. It is possible that the veins will need to be removed if the venous insufficiency has progressed to a very advanced stage and the veins have been severely damaged. This kind of removal is often referred to as "vein stripping" (Rigby et al., 2013; Ombrellino & Kabnick, 2005).

1.6.7. 4. ***Surgical repair.*** In this case, surgical treatments are utilized in order to make the necessary adjustments to the dysfunctional venous valves. There are numerous approaches to achieving this goal, but the valvuloplasty procedure, in which the valve is tightened and mended with stitches, is by far the most common (Dalsing & Kistner, 2019; Balaram & Bassin,2023).

1.6.7.5. Subfascial Endoscopy. If the injured veins are perforator veins, then venous insufficiency may be treated with a surgical operation called perforator surgery. Veins known as perforator veins connect the body's superficial veins with its deep veins. The calf will be punctured with a few tiny holes, and the perforator veins will then have

surgical clips applied to them to close them off (Kalra & Gloviczki, 2003; Kalra et al., 2002).

Vein bypass surgery is rerouting blood flow by diverting healthy veins from one part of the body to another and surgically transferring them to an area of the body with veins that are suffering from venous insufficiency. This process is known as "vein harvesting." Blood is "transplanted" to the new veins once they've been relocated, so that they can begin functioning normally. One of the most popular treatments for venous insufficiency is a procedure called a vein bypass. This course of action is usually not taken unless all other treatments have been tried and found to be unsuccessful (Tiwary & Katiyar, 2022).

1.7. Statement of the problem

Cardiovascular diseases (CVDs) are the leading cause of death globally, taking an estimated 17.9 million lives each year (Acker-Gussmann, Annette, and Renate Oberhoffer-Fritz 2022). CVDs are a group of disorders of the heart and blood vessels and include coronary heart disease, cerebrovascular disease, rheumatic heart disease and other conditions **(Meng,** Jia, and Baoxue Yang, 2019**).** More than four out of five CVD deaths are due to heart attacks and strokes, and one third of these deaths occur prematurely in people under 70 years of age 3 (Zhang, Jun, et al 2020). Cardiovascular diseases (CVDs) are responsible for nearly half (47%) of all deaths in Turkey. By 2030, CVD mortality is projected to increase ≈2.3-fold in men and 1.8-fold in women (Kayikcioğlu, M., & Oto, A. 2020).

1.8. The Purpose of this study

The goal of the research was to analyse the expression pathways of *IL-8* and *IL-18* genes in veins as well as in aorta tissues that were obtained from patients suffering from a wide range of cardiovascular conditions. It is hypothesized that the over expression of one of the particular gene is one of the factors determining whether an individual will develop cardiovascular disease (CVDs).

1.9. Research Questions and Hypothesis

1.9.1. Hypothesis

($H_{a\ -1}$) Expression level has a significant impact and association with cardia vascular disorder and there is a significant statistical difference in the expression between control group and patients group.

($H_{o\ -1}$): There is no statistically significant difference between the expression level in the control group and the patient group and that the expression level has no association with cardia vascular disorder

($H_{a\ -2}$) There is significance statistical difference between the expression level of IL 8 and IL 18 in veins and Aorta

($H_{o\ -2}$): There is no statistical significance between the expression level of IL 8 and IL 18 in vein and Aorta.

1.9.2. Research Questions

1. What is the expression level of *IL-18* in the veins of healthy people and affected individuals?
2. What is the expression profile of IL 8 and IL 18 in veins and Aorta Tissue?
3. What is the expression of *IL-8* in the aorta of the control group and the expression of *IL-8* in the group of patients

1.10. The significance of this study

This study is significant in that it provides reliable and additional scientific data on the expression profile of interleukins 8 and interleukin 18 in veins and aorta. Getting an understanding on how interleukin 8 and interleukin 18 are expressed is a major advancement for further studies, this could lead to the regulation and prevention of CVDs and other diseases in pregnant women, new born and elderly.

1.11. Limitations

In general, expression profiling studies report those genes that showed statistically significant differences under changed experimental conditions. This is typically a small fraction of the genome for several reasons. First, different cells and tissues express a subset of genes as a direct consequence of cellular differentiation so many genes are turned off. Second, many of the gene's code for proteins that are required for survival in very specific amounts so many genes do not change. Third, cells use many other mechanisms to regulate proteins in addition to altering the amount

of mRNA, so these genes may stay consistently expressed even when protein concentrations are rising and falling. Fourth, financial constraints limit expression profiling experiments to a small number of observations of the same gene under identical conditions, reducing the statistical power of the experiment, making it impossible for the experiment to identify important but subtle changes. Finally, it takes a great amount of effort to discuss the biological significance of each regulated gene, therefore the discussion is limited.

1.12. Definition of terms

Gene expression: Gene expression is a tightly regulated process that allows a cell to respond to its changing environment. Both an off and on switch to control when proteins are made and also a volume control that increases or decreases the amount of proteins made.

Interleukin-8: interleukin 8 is a chemokine produced by macrophages and other cell types such as epithelia cells airway smooth muscles cells and endothelial cells.

Interleukin-18: Interleukin 18, also known as interferon-gamma inducing factor is a protein which in human is encoded by the IL 18 gene.

Veins: veins are blood vessels located throughout the body that collect oxygen –poor blood

Aorta: The Aorta is the largest artery of the body and carries blood from the heart to the circulatory system.

Cardiovascular disease: Cardia Vascular Disease (CVDs) are general terms that describes a disease of the heart or blood vessel.

CHAPTER II:

Literature Review

Research related conceptual definitions, descriptions and information related to the subject that already exists in the literature are given in this chapter.

2.1. Theoretical Framework

Accumulating evidence suggests that inflammation plays an essential role in the pathogenesis of atherosclerosis and that circulating inflammatory markers predict future cardiovascular events (Alfaddagh, Abdulhamied, et al 202).

Recent researches have suggested that various inflammatory mediators play a causative role in several steps involved in the progression of atherosclerosis from local inflammation through plaque formation and rupture (Alfaddagh, Abdulhamied, et al 2020). Amongst these mediators, various cytokines, including inflammatory cytokines and anti-inflammatory cytokines participate in the inflammatory process **(**Sterpetti, A.V, 2020).

2.2. Related Research

Research by Ji et al., (2021) has shown that interleukins are involved in human “myocardial infarction”, heart failure, and other heart-related issues. High serum levels of interleukin-eighteen were associated with an increased risk of “cardiovascular disease” in individuals with heart failure, “acute myocardial infarction”, and “acute coronary syndromes”, as well as in the general populace, as reported by O'Brien et al. (2014). “Acute coronary syndrome” patients were at an even greater risk of acquiring cardiovascular disease. Chai et al., (2022) found a link between high levels of “atrial natriuretic peptide” and high levels of "interleukin-eighteen" in the plasma of people with "acute myocardial infarction”. This suggests that "interleukin-eighteen" may have a part to play.

It would appear that free “interleukin-eighteen” levels coincide more closely with disease activity than total “*interleukin-eighteen*” levels do because naturally occurring "IL-18BP" in the circulation works to neutralize the circulating “*interleukin-eighteen*” resulting in much lower "free" “interleukin-eighteen” levels than total “interleukin-eighteen” levels. This is because “*IL-18BP*”, which is produced naturally, counteracts “interleukin-eighteen” in the bloodstream. Specifically, free levels of the

cytokine “interleukin-eighteen” seem to be more predictive of the severity of the illness (Hareletal, 2020).

According to the findings of a study that used biopsy and post-mortem samples from patients who had the illness, an inflammation was identified in the cardiac tissue of individuals who had acute myocarditis, but it was not found in the heart tissue of participants who served as controls for the study (Ferrer-Gómez et al., 2021). In addition, the research demonstrated a link between the severity of HF symptoms and the existence of the “inflammation” in the heart, which indicated a lack of functional recovery during the follow-up period. The analysis provided evidence for this assertion. The information that has been presented suggests that the “inflammation”, in addition to the related cytokines like *“IL-1 and IL-18”,* may have a major impact on the tissue injury response that is generated by myocarditis.

In yet another study, *“interleukin-eighteen*”, levels were studied with those of its chains and binding protein in rats who had “auto-immune myocarditis”. The levels of all the proteins were found to be significantly higher in myeloid cells, whereas cardio myocytes only showed a slight increase.

It has been shown that the *“IL-8, CXCR1*”, and “*CXCR2* genes” are more active in people with persistent perfusion problems (Leonardetal, 2011). As fibrosis and "cardio myocyte hypertrophy" progress, heart failure with preserved ejection fraction (HFPEF) can make it difficult for the left ventricle (LV) to fill up completely. This is due to the fact that the heart is still able to pump blood (Rocca, 2022; Schwinger, 2021). "IL-18" is a contender for being a target for HFPEF because of the “pro-hypertrophic” and “profibrotic” actions that it carries out. (Chandrasekar et al., 2005).This is because it plays a part in the pathophysiology of the condition, which makes it a candidate for being a target.

O'Brien et al., (2014) found that prolonged *"IL-18* blocking" has the potential to halt or even repair the progression of pathologic ventricular hypertrophy. Though chronically "pro-hypertrophic and profibrotic" in animal studies, "*IL-18*" may also prevent "physiologic hypertrophy" from developing.These effects were observed when the researchers performed “IL-18 blocking” on animal’s heart.

Heart failure (HF) is a medical condition that causes the left ventricle to work less well, cause shortness of breath, fatigue, and a low tolerance for exercise. An electrocardiogram (ECG) and a physical examination can both identify HF.

According to Philipou et al., (2020), it is estimated that 64.3 million individuals around the world are currently living with some form of heart failure. The incidence of recognized heart failure is commonly considered to be anywhere from 1 percent to 2 percent of the general adult population in affluent nations (Groenewegen, 2020; Disease, 2018). Although patient survival has increased in recent years, it is still far too low; more than half of those who find themselves with heart failure will succumb to it within the first five-year period after being diagnosed (Tomasoni et al., 2020). Inflammation, which promotes changes in the "extracellular matrix," cell growth, "cardiomyocyte hypertrophy," and "cardio myocyte" contractions in response to tissue stress and injury, coordinates heart tissue repair and recovery in part. Inflammation causes this by triggering the body's inflammatory response. The ability of inflammation to provoke an inflammatory response is responsible for this (Bowers et al., 2022). While inflammation plays a function in the body's natural repair mechanisms, too much of it appears to increase the risk of developing cardiovascular disease. It may also affect the disease's progression and response to treatment. While inflammation plays an important part in the body's natural ability to repair itself, studies have linked it to an increased risk of cardiovascular disease (Anderton et al., 2020).

The seriousness of cardiac dysfunction (HF) and the likelihood of a poor outcome are both correlated with the level of inflammation measured by biomarkers (Takagi et al., 2023). In controlled laboratory settings, proinflammatory cytokines are shown to cause dysfunction in the left ventricle (Anzai et al., 2022). On the other hand, anti-inflammatory treatments for HF are currently insufficient, which indicates that the inflammatory processes that are implicated in the development of HF are not fully understood. Neutrophil proteinases on the outside of the cells, like neutrophil proteinase 3 (PR3), could turn on *interleukin-18 (IL-18)* when damaged tissue is present (Dubyak et al., 2023). Even though the majority of *IL-18* exists in a soluble form, there is a possibility that some macrophages contain it in a membrane-bound form. These cells will release soluble "IL-18" when they have been activated with "lipopolysaccharide (LPS)". This shows that there is another pathway for the release and activation of "IL-18" by an LPS-induced protease, most likely PR3 (Yasuda et al., 2019). It is possible that PR3 is the mechanism that triggers "*pro-IL-1* and *pro-IL-18*," which are produced by dying cells, and that there is another pathway for the release

and activation of "IL-18" by an LPS-induced protease, most likely PR3. (Yasuda et al., 2019).

Conditions as diverse as acute "myocardial infarction" and heart failure have been linked to elevated IL-18 activity. It is reasonable to hypothesize that "IL-18 blockade" could be an effective technique in both acute and chronic cardiac disorders, given the role that "IL-18" plays in mediating acute cardiac consequences such as contractile failure and AR desensitization. This is due to the fact that "IL-18" mediates the effects of stress on the heart (Vasques-Nóvoaetal., 2018). Contractile function can be improved and the size of an infarct can be reduced in patients who have had an" ischemia-reperfusion" injury if the activity of "IL-18" is blocked with a “neutralizing antibody”, "IL-18BPa, NLRP3" inhibitors, or caspase-1 inhibitors. These treatments are administered to individuals who have experienced an ischemia-reperfusion injury. Because of this, further investigation into the role that these chemicals play in AMI is now possible (Aydin et al., 2019). Inhibiting *IL-18,* on the other hand, may have the unintended consequence of hindering the body's natural hypertrophic response to pressure stress, which in turn may lead to maladaptive remodelling (Lvetal, 2022). In preclinical settings, there have been no trials of *IL-18* targeted therapy with "IL-18BP or IL-18Ab," administered either during or after reperfusion, and these treatments have not been tested in ischemia. If these investigations reveal that *"IL-18"* has a protective effect that lasts over time and is not connected to slower healing of the infarct in preclinical AMI models, then it may be required to conduct pilot clinical trials in both "ST-segment elevation AMI (STEMI)" and non-STEMI patients. IL-1 blockers have been used in the past to accomplish something similar to what is being described here (O'Brien et al., 2014).

In AMI, an analysis of "interleukin-eighteen and one" blockers may provide further insight into whether the two "cytokines" have parallel or combinatorial effects or if interleukin-eighteen facilitates the effects of interleukin-one.This could be useful in determining which of these possibilities is true. This may be the case if one of the *“cytokines, IL-18*”, mediates the effects of the other cytokine, *IL-1* (Detryetal, 2022). Since *“IL-18”* has a strong cardio depressant effect, blocking its activity may be a good way to treat heart failure (HF) that is acutely decompensated or has persistent symptoms (Wang et al., 2022).It looks like “interleukin- eighteen affects both how the heart contracts and how it relaxes (Xing et al., 2010).

Studies on inhibiting "interleukin-one" have shown promising results in patients with HF (Van Tassell et al., 2015), and the biological links connecting "interleukin-eighteen" and "interleukin-one" imply that blocking "interleukin-eighteen" may have equivalent positive effects (Cavalli et al., 2021), as a result, blocking “interleukin-eighteen” can enhance LV function in people with high levels of *IL-1* without affecting the other effects that *IL-1*mediates. There are numerous studies that have been published in the academic literature that point to a possible connection between the *“interleukin 8* and *interleukin* 18” pathways and vascular diseases. However, additional research needs to be done in order to provide data on the expression profiling of interleukin 8 and interleukin 18 in veins and the aorta. This research could provide more information regarding these cytokines, particularly in the veins and aorta.

Interleukins play a significant part in the formation of newly formed blood vessels, an event that is initiated by numerous growth factors and cytokines that may act directly or indirectly in a variety of different ways (Kang & Kishimoto, (2021).

Macrophages are primarily responsible for the production of “IL-18”, which can also be made by hepatocytes and keratinocytes. Its primary objective is to inhibit a co-factor that is necessary for the induction of Th1 cells. It does this by causing the production of interferon gamma and by enhancing the activity of NK cells (Vaillant, 2022d). According to the findings of Satş et al. (2021), *“IL-18”* levels are directly link with “coagulation parameters” as well as “renal, hepatic, and cardiac” injury markers, which suggests that it may contribute to pathologic multiorgan injury. According to the findings of a study that was carried out by (Bouabdallah et al. 2019), *IL-8* is the most powerful independent predictor of both cardiovascular and all-cause events.

The components of the extracellular matrix are responsible for determining the tissue's mechanical properties, striking an equilibrium between rigidity and flexibility. This occurs in all blood vessels (Andreeva et al., 2022). Variations in the rigidity and flexibility of vein walls could therefore be caused by shifts in the “expression” and production levels of “extracellular matrix” components. This could be one of the factors that leads to the formation of “venous insufficiency” in “primary varicose” veins. The presence of swollen veins that bulge outward from the surface of the body is diagnostic of varicose veins. Varicose veins are also known as spider veins. According to the findings of a number of investigations (Crisan & Crisan, 2022) and previous studies (Pai et al., 2021), Inflammation has been shown to have a significant

impact on the onset of limb-threatening deep vein thrombosis. Interleukin-1 beta is a type of inflammatory cytokine that is crucial in the onset of inflammation throughout the body and in specific areas.

According to Wooff et al. (2019), it is also a marker of an early inflammatory response in the body. Kim et al. (2005) examined the larger “saphenous” veins of patients who had “varicose” veins and then contrasted these veins with the larger “saphenous” veins of people who did not have “varicose” veins. The analyzed cDNA clones were found to be useful disease markers in the genetic diagnosis of primary varicose veins, and the expression patterns of the L1 and Alu elements in genes encoding structural proteins suggested a role for these elements in the pathogenesis of primary varicose veins. Hepatocytes and keratinocytes can also produce "IL-18," but macrophages produce the majority of it (Vaillant, 2022).

Induction of Th1 cells relies heavily on “IL-18”, both as a primary target and as a co-factor. It does this by causing the production of interferon gamma and by enhancing the activity of NK cells. IL-8 is produced by monocytes as well as fibroblasts. The neutrophils, basophils, mast cells, macrophages, and keratinocytes that are most susceptible to its effects are its primary targets. Neutrophil chemotaxis, angiogenesis, the release of superoxide, and granule release are all caused by it.

In yet another study, Pai et al. (2021) investigated the expression and role of “inflammatory factors in DVT”, they came to the conclusion that there were no significant differences in the serum levels of IL-1, TF, and XOD between the groups that underwent sham surgery and those that underwent control procedures. However, those in the rat model of DVT showed an increasing trend from two to twenty-four hours and peaked at twenty-four hours, with a significant difference from the comparable levels in the control and sham surgery groups. This was the case even though the sham surgery group was not subjected to real surgery. This was the situation throughout the entirety of the research investigation. Throughout the entirety of the investigation, this was consistently the case.

In their study S. Lee et.al. (2005), they used complementary deoxyribonucleic acid (cDNA) microarrays to look into the differentiation of gene expression in the wall of the VV. They discovered that eighty-two genes had their expression levels increased in VVs. cDNA microarrays were able to identify some of the genes whose expression levels had increased, such as the transforming growth factor–induced gene (BIGH3), tubulin, lumican, actinin, collagen type I, and versican. They reached this conclusion

by analyzing the data from a cDNA microarray and finding that VVs comprised many genes with elevated expression levels. These gene profiles provided evidence for the existence of a pathway linked to fibrosis as well as the potential that wound healing is connected to the "pathophysiology" of "VVs." It is possible that "polymorphisms" in genes which play a role in the production of "cytokines" might have significant function in the progression of certain diseases. Explain, at least in part, why people of different races have varying rates of "ASCVD" risk. The researchers, Van Dyke et al., looked at one hundred and three "African American" women and three hundred eighty "Caucasian women" and discovered that there were differences between the races in fifty-two single "nucleotide polymorphisms" that were related to significant inflammatory pathways. The study was conducted on women of both races. According to these findings, the genetic expression of “pro-inflammatory” *"IL-1 gene*" is found to be higher in African Americans, but “expression of the anti-inflammatory” "IL-10 gene" is found to be lower in "African Americans" in comparison to Caucasians (Alfaddagh et al., 2020).

In addition, a genome-wide association study that was carried out using 3109 African Americans and 6,000 "European Americans" discovered that "ApoE-2 rs7214 single-nucleotide polymorphisms" in "African Americans" are related to higher levels of CRP when compared with "European Americans." This finding was made possible by the fact that “African Americans” had a larger sample size than European Americans (Robinson et al., 2012).

When you look at the results of population-based genetic association studies as a whole, you can see that there are big differences between people in terms of the levels of biomarkers in their blood. In addition to this, genetic polymorphisms may also play a role in figuring out how well measuring circulating cytokines can predict what will happen. It has also been reported among other things that single gene "polymorphisms" have a modest effect on the heritability of traits as a whole as well as the levels of "cytokines."(Zhang, 2023; Polfus, 2019). As a result, the contribution of single genetic polymorphisms to the overall risk of cardiovascular disease is very small. When genetic polymorphisms are understood, it may be possible to shed light on possible mechanisms that may explain why some people have different responses to targeted “anti-inflammatory therapies”. For instance, in the “CANTOS trial”, some of the people who were randomly assigned to receive canakinumab did not experience a reduction in their levels of high-sensitivity CRP (Alfaddagh et al., 2020n). At this

point, it was unclear if certain genetic traits had anything to do with the different treatment effects that were seen.

CHAPTER III:

Methodology

This chapter presents all the material and methods that were used in the conduct of the research. It provides a step by procedures and description of test kits and reagents used for the DNA extraction, cDNA synthesis, gel electrophoresis and PCR reactions.

3.1. Research Designed

A research design is a thorough blueprint of a research undertaking. It generally comprises the data collection process, the instruments that will be used, and how these instruments and data will be processed to get a valid conclusion on the research issue. "A study design is a plan that guides the investigator in the process of collecting, analyzing, and interpreting observations," writes (Yin, 2014). "It is a logical proof model that allows the researcher to draw inferences about causal relationships among variables under inquiry" (Taole, 2008).

This research design aims to investigate the expression profile of interleukin 8 and interleukin 18 in veins and aorta tissues cells extracted from patients in Northern Cyprus. The study utilizes a quantitative approach, making used of laboratory analysis to generate data on the gene profile.

This research follows the usual chronological six-part framework. The context and problem statement, significant of the study, objective of the study, study questions, and study limitations were defined and explained in detail in the first phase of the introduction. Furthermore, the methodology and data analysis have been described in depth in accordance with the sequence of the research. The study was completed by including a discussion session for additional research and briefly addressing the constraints identified during the investigation

3.2. Participants/ Population & the Sample/ Study group

The patient and sample categories are shown in the table 3.1 and figure 3.1 below. The samples are divided into control and patient categories, the normal veins were used as the control for the vein samples and the cell types are veins and aorta tissues cell.

Samples from the veins and aorta tissue of one-hundred three (103) individuals who had been diagnosed with a variety of cardiovascular conditions were taken from patients by excision of a saphenous vein / aorta as varicose treatment coronary bypass operation. These samples were examined to determine the expression pathways of *interleukins 8* and *interleukin 18* in veins and aorta tissues. Samples were taken in two separate batches and assigned to either the control group or the patient group. In the patient group, a total of thirty two (32) vein samples and thirty (32) aorta samples were taken, which adds up to 64 patient samples.

In the control group, on the other hand, 11 abnormal veins and 28 normal veins which were considered control samples were taken, this brought the total number of control samples to 39. The samples taken from the veins accounted for 43% of the total samples collected, whereas the samples taken from the aorta accounted for 60% of the samples taken as stated in the table below in table 3.1 and figure 3.1

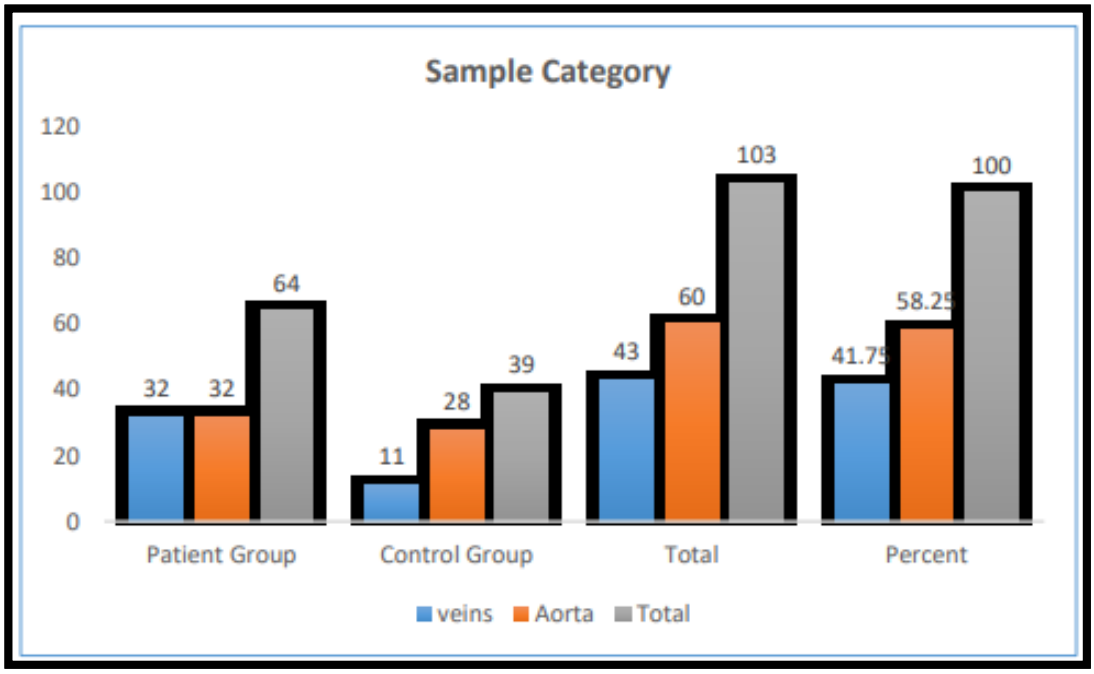
Table3.1

Sample category

Sample type	Patient group	Control group	Total	Percent
Veins	32	11	43	41.75
Aorta	32	28	60	58.25
Total	64	39	103	100

Figure 3.1

Sample category



3.3. **Data collection tools/Materials**

3.3.1. Suppliers

Thermo-scientific marker (Pittsburg, USA), Nano-drop (Thermo-scientific, Pittsburg, USA), cDNA Synthesis Kit (Basel, Switzerland). Applied bio-systems thermal cycler PCR, (Waltham, Massachusetts, USA), Eppendorf Scientific (Hamburg, Germany), RotarGene Real-Time PCR (Qiagen, Hilden, Germany), Bio-Rad Electrophoresis instrument (Hemel Hemstead, UK), Ultraviolent Trans-Illuminator (DNR Bio-Imaging System, Neve Yamin, Israel).

3.3.2. Chemical Reagents

3.3.2.1. Standard Solutions. In accordance with Sambrook ET al.1989 instructions, a "10X Tris-borate/EDTA (TBE) electrophoresis buffer" was prepared. After that, it was diluted some more until it reached a concentration of "1X (100 ml of 10X TBE plus 900 ml of distilled water)". It slows the bands' movements because it is necessarily concentrated, the "10X TBE buffer" needs to be diluted before it can be used. "Thermo-Scientific 2x Master" Mix was the second solution. This solution contains 0.05 units per "microliter of Taq DNA polymerase", reaction buffer, 4 nanometers of magnesium chloride, and 0.4 nanometers of each" dNTP (dATP, dCTP, dTTP, dGTP). Wiz-Pure qPCR SYBR Green" served as the third solution (Seongam, South Korea).

This particular “SYBR green includes antibody-mediated hot star”, “Taq DNA polymerase”, “ultrapure dNTPs”, “MgCl2”, and “SYBR green I”, which has both enhancers and stabilizers.

3.3.4. Other chemical agents

“Agarose biomax 100mg, Ethidium Bromide (Serva, Heidelberg, Germany)”

3.3.5 Molecular Weight Markers

Thermo-scientific 50 bp – 1000 bp (Pittsburg, USA) DNA ladder was utilized as a molecular weight marker.

3.3.6. Oligonucleotides

The primers used were manufactured by Oligomer Company (Turkey)

Table 3.2

Reversed and forward primers for IL 8 and IL 18

Interleukin 18 (IL 18) Transcript Variant 1 : NM 00156.4
Forward : CAGATCGCTTCCTCTCGCAA
Reversed : CCAGGTTTTCATCATCTTCAGCTAT
C-X-C Motif Chemokine Ligand 8 (CXCL 8), Transcript variant 1 (IL 8) (NM000584.4)
Foward : GAAGTTTTTGAAGAGGGCTGAGA
Reversed : ACCAAGGCACAGTGGAACAA
House keeping gene : ACTB (Belta Actin)

3.4. Data collection procedures

3.4.1. Process diagram

The following pattern were implemented for the conduct of the research as seen in figure 3.2.

Figure 3.2

Process diagram show the research process

3.4.1. Sampling Techniques and Sample collection

A sample is a group of a relatively small number of persons taken from a population for research purposes" (Alvi, 2016). Samples were collected from the CVF facility and laboratory of the Near East University Hospital (NEUH) after the consent of Near East University Scientific Review Board (registration number: YDU/2023/112-1706.). Samples of veins and aorta tissue were collected from individuals who had been diagnosed with a variety of cardiovascular conditions. Samples were taken from patients by excision of a saphenous vein / aorta as varicose treatment coronary bypass operation. These samples were examined to determine the levels of expression of interleukins 8 and interleukins 18 in tissues and aorta cells.

Samples were taken in two separate batches and assigned to either the control group or the patient group.

The control group consisted of samples taken from individuals who were in good health but have associated risk factors, normal veins were taken as control veins. The "Near East University DESAM Institute Molecular Medicine Laboratory" in the northern part of Cyprus, was the location where the preliminary research for this project was conducted. In order to eliminate any possibility of the experiment being tainted by contamination, each of the reagents, pipettes, and tubes used in it were UV-treated.

3.5. Data Analysis plan

3.5.1. Analysis of Gene Expression

For the *IL-8 and IL18* and the house keeping genes, synthesized cDNA was used to look at how the genes were expressed. RT-qPCR was used to conduct the gene expression analysis; this technique allows for the observation of a positive reaction through the accumulation of fluorescent signal. The number of cycles that must pass before the fluorescent signal is considered to have passed the cycle threshold, also known as Ct, the experiment was carried out at the ideal “annealing temperature” of 57℃ for an hour and a half.

The “RT-qPCR analysis” was performed on all 103 samples, including the control groups. The resulting Ct values were calculated and the 2^-ΔΔCT value was used to calculate the gene expression value in vein and aorta tissues as seen in the table below, The resulting Ct values were calculated and the 2^-ΔΔCT value we were able to determine the gene expression value in vein and aorta tissues.

3.5.2. Statistical Analysis

In the past few years, processing microarray data has become a topic that researchers are very interested in (Abd-Elnaby et al., 2021). The comparative 2-ΔCt approach was applied for the purpose of relative quantity measurement. (Livak & Schmittgen, 2001). In order to do the analysis on the data, graph pad was utilized. The three-way ANOVA repeated analysis were utilized to conduct the analysis of the differences that emerged within a single group as a function of expression.

3.6. RNA Extraction from specimens

The RNA from the veins and aorta samples were extracted using the Hibrigen genomic RNA isolation kit by Thermo scientific and following the manufacturer instruction.

3.6.1. Measuring RNA concentration

The "optical density at 260/280" nm was measured using a nano-drop spectrometer (Thermo-Scientific, Pittsburgh, USA), which gave an estimate of the concentration and purity of the "RNA" sample. For best results, the RNA should be purified to a density of about 2.1 ng/l. From the results obtained, the RNA purity and concentration were all satisfactory and ideal for the purpose of the research.

3.6.2. Complementary DNA (c DNA) synthesis

The first-strand cDNA synthesis kit from Transcriptor was used to create cDNA. (Basel, Switzerland). An anchored oligo dT18 primer, a dNTP mixture, Trascriptor reverse transcriptase, and Trascriptor RT reaction buffer were all included in this kit. A 5x concentrated RNAse inhibitor "was additionally added. A random hexamer primer that includes both forward and reverse primers is also included. All of these elements were mixed with 2µl of RNA that had been stored between -15°C and -20°C.

Table 3. 3

Calculations for "cDNA synthesis"

Components	1 X measurement
Rxn Buffer	2.0µl
Random hexane	2.0 µl
dNTPs	1.0 µl
RTase	3.50 µl
RNase	10.0 µl
Total	18.50 µl

3.6.3. Primer Optimization for Gradient PCR

In order to proceed with the primer optimization phase of this research project, we first needed to generate "oligomer stock primers" for the two genes. It is necessary to add a specific quantity of distilled water to each gene primer in order to bring the total concentration up to 100 mM. After that, 10 microliters of "stock primer" and 90 microliters of distilled water are mixed together to create a working solution with a concentration of 10 milliMolar. As a result of this, the concentration is now 10 mM.

Table 3.4

Stock primers of Interleukin 8 and Interleukin 18 genes

Oligo name	Base Sequence 5' 3'	100µM Stock µl TE
IL 18-1-F	CAGATCGCTTCCTCTCGCA	476
IL 18-R	CCAGGTTTTCATCATCTTGAGCTA	588
CXCL 8 (IL 8) - F	GAAGTTTTTGAAGAGGGCTGAGA	497
CXCL 8 (IL 8)-R	ACCAAGGCACAGTGGAACAA	544

In order to conduct the gradient PCR, the appropriate "Bio Systems Vertiti 96-well thermal cycler" (Waltham, Massachusetts, USA) was used. The quantitative real-time PCR was employed for differentiating the optimal temperature. Both genes went through this process. The desired temperature range was 57°C to 95°C. The gradient PCR settings that were used are listed in Table 3.6 below and the total analysis time was roughly one hour and thirty minutes. Each and every one of the" PCR reactions", as well as the A "category II laminar flow hood" was used for "RT-qPCR" experiments to prevent cross-contamination. Each and every one of the reagents, together with the plastic ware and pipettes, was sanitized and marked with its PCR affiliation.

3.6.4. Primer Optimization for qRT- PCR

Both the "RotarGene Real Time PCR" and the RT-qPCR (Qiagen, Hilden, Germany) were used to detect and quantify the PCR products generated in each cycle. Similar to gradient PCR, several calculations were performed to determine the exact amounts of each reagent needed to analyze 103 samples plus two NTC for each batch of the three genes. For the purpose of quantitative analysis, 19.0µl of the final mixture and 1.0µl of Complimentary DNA were added to PCR tubes manufactured by Eppendorf Scientific in Hamburg, Germany. This brought the total volume of the reaction up to 20.0µl. The analysis took approximately 1 hour and 15 minutes to complete

Table 3.5

RT-qPCR Master Mixture calculation .

Components	1X
Master Mixed	12.5 µl
Forward	1.25 µl
Reversed	1.25 µl
DNA free water	9.0 µl

The amplification of cDNA was conducted in a laminar flow wood which was first sanitized with 95% alcohol (v/v) to reduce contamination. All the equipment were also cleaned to limit contamination. The amplification of cDNA was performed to produce exponential copies of cDNA. All tubes where properly closed, labeled and placed in a thermo cycler PCR programmable amplification machine which ran for 35 cycles using the three main steps in PCR amplification. Denaturation stage occurred for 1 minute at 95^0C, annealing stage occurred for 30 second at a temperature of 57^oC and finally extension stage was at a temperature of 72^0C in 1 minutes as seen in table 3.6.

Table 3.6

Quantitative real time PCR condition at 40 cycles

Stage	Temperature	Duration	Cycle
Initial Denaturation	95	1 minutes	1
Denaturation	95	15 seconds	
Annealing	57	30 seconds	40 cycles
Extension	72	45 seconds	

3.6.5 "Agarose gel Electrophoresis"

The obtained products from the gradient PCR were analysed using gel electrophoresis once the PCR was completed. With the help of Sigma agarose, a gel with a concentration of 2% (w/v) was made. "Merck KGA-A, "Darmstadt, Germany" 2.40 grams of agarose powder was mixed with 120 ml of "TBE buffer" measuring. It was pulled out of the microwave after being there for thirty seconds at the highest power level, given a brief stir, and then put back into the microwave. This process was repeated three times. Following a number of iterations, the technique was carried out until the mixture hit the boiling point and turned clear. After that, it was put away for one to two minutes so that the heat could dissipate more gradually. Following the addition of 0.25µl of ethidium bromide to the mixture and its subsequent thorough mixing, the substance in question was then put into a tray that measured 20 X 20 centimeters. After the gel mixture had been put into the tray, it was left alone for some time in order to allow it to solidify. After thoroughly combining 6.0µl of each PCR product with 2.0µl of loading dye "Thermo Scientific, Pittsburgh, United States", the resulting mixture was placed into the wells for analysis. In the end, a ladder of a known size was loaded along with the samples, taking up a total of 2.0µl. The "Bio-Rad electrophoresis apparatus" was used to run the samples at voltages ranging from 130 to 140 volts. "Hemel Hemstead, UK". To accomplish the work, it took around 1 hour

30 minutes. The bands were able to be seen thanks to the utilization of a UV transilluminator. “DNR Bio Imaging System, Neve Yamin, Israel”.

CHAPTER IV:

Findings and Discussions

This chapter presents the findings based on the collected data.

4.1. Findings for research Questions

4.1.1. Findings for research Question I

The expression of *IL-18* in the veins of healthy people is significantly higher than that of affected individuals, with a P value of 0.0151 ($P<0.05$).

4.1.2. Findings for research Question II

"IL-18 and IL-8" are significantly expressed in venous tissues than in the aorta tissues.

4.1.3. Findings for research Question III

When comparing the expression of *IL-8* in the aorta of the control group and the expression of *IL-8* in the group of patients, we find that the expression of *IL-8* in the aorta of the control study group is statistically significant and significantly greater than the expression of *IL-18* in the vein tissues of the control study group (P-value $=0.0158$, $P<0.05$).

4.2. Discussions

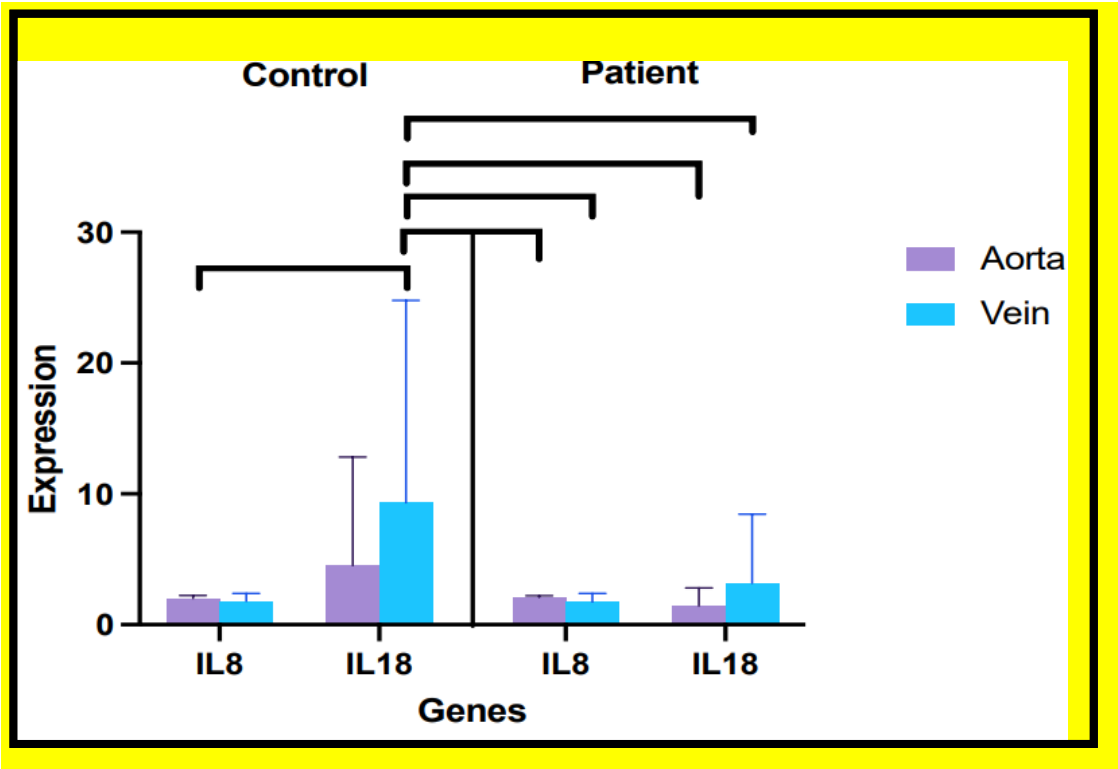
As described in figure 4.1 below, With a P-value of 0.0151 the IL-18 concentrations found in the veins of the control group is substantially higher than the IL-18 expression found in the veins of the patient groups. In addition to this, the level of IL-18 expression in the aorta of the patient group is noticeably higher than the level of expression in the veins of the patient group with a P-value of 0.0015 and while the expression of IL-18 patients' aorta is significantly higher that IL-18 expression in the control veins with a P-value of 0.0003.

The expression of IL-8 in the veins of the control group is identical to the expression in the veins of the patient group, with a P-value of 0.0008 And while the expression of IL-8 in the aorta of the control group is the same as the expression of IL-8 in the group of patients, In comparison, the levels of IL-8 expression in vein and aorta, the level of IL-8 expression in aorta tissues of the control study group is statistically significant

and significantly greater than the level of IL-18 expression in vein tissues of the control study group, as indicated by a P-value of 0.0158 ($P<0.05$). And that the IL-8 expression level in the patient study group's vein tissue is statistically substantially lower than the IL-8 expression level in the patient study group's aorta tissue.

Figure 4.1

Showing correlation between the expression of genes in various tissues



The graph demonstrates that the expression of level of *IL-18* of control group of veins and aorta tissue cell is significantly higher than the expression of *IL-18* in aorta tissue cell. On the other hand, the expression of *IL-18* in the patient group of both veins and aorta tissue cells shows that vein is statistically significantly expressed than aorta tissue cell. As a result of further comparison, it is clear that the level of *IL-18* expression in the control group is noticeably higher than that of the patient group. In other words, *IL-18* is elevated in the group that served as the control, whereas it is downregulated in the group that served as the patient. And although while the expression level of *IL-8* did not show any statistically significant differences between the control group and the sick group, this does not mean that there was no change.

4.3. Conclusion

Using tissue samples from 103 individuals, we studied the expression pathways of *interleukin* 8 and *interleukin 18* in veins and aorta cells and found a statistically significant and correlative relationship between the two genes and the two tissue cells. It was determined that there was no statistically significant difference in *IL-8* expression between the control group and the experimental group of vein and aorta tissues. In contrast*, IL-18* expression in vein and aortic tissue cells in the control group was found to be considerably higher in vein tissue cells than in aorta tissue cells. Furthermore, there is no statistically significant expression of *IL-8* in either vein or aorta tissues among the patient group. However, statistical analysis of *IL-18* expression in veins and aorta tissue cells from the same patient group reveals that *IL-18* is significantly expressed in veins. A closer examination reveals that the control group expresses significantly more *IL-18* than the sick group. To rephrase, *IL-18* was found to be increased in the control group and downregulated in the sick group. Although there were no statistically significant differences in *IL-8* expression levels between the healthy controls and the sick patients, this does not rule out the possibility of change. This data lends credence to the assertion that *IL-18* is responsible for immunity and defence, demonstrating that the patient group was not adequately protected due to the fact that the expression of *IL-18* downregulated in the patient group. The result also demonstrates that venous tissues are suitable tissues for research of interleukins to be conducted on as compare to aorta tissue

CHAPTER V:

Discussion

The findings of this study shows that *IL-18*, a pro-inflammatory cytokine, expression levels in the control in the control group of veins and aorta tissue cell indicates that *IL-18* in vein tissue cell is significantly higher than the expression of *IL-18* in aorta tissue cell. Additionally, the expression of *IL-8* in the patient group of both vein and aorta tissues does not have any statistically significant expression. On the other hand, the expression of *IL-18* in the patient group of both veins and aorta tissue cells shows that vein is statistically significantly more highly expressed than aorta tissue cell. As a result of further comparison, it is clear that the level of *IL-18* expression in the control group is noticeably higher than that of the patient group. In other words, *IL-18* is elevated in the group that served as the control, whereas it is downregulated in the group that served as the patient. And although while the expression level of *IL-8* did not show any statistically significant differences between the control group and the sick group, this does not mean that there was no change.

Recent research has indicated that "inflammatory diseases and cardiovascular diseases (CVD)" have a significant link to one another and should be treated as a single entity (Sapp, 2020; Vaduganathan, 2022). It is crucial to uncover critical regulatory mechanisms in order to increase our understanding of the disease. Because of this, there is a possibility that fresh “therapy” alternatives will be developed in the future, which will assist in lowering the mortality rate that is linked with the ailment. Because of a number of important discoveries that have been made, the *"IL-8"* and *"IL-18"* pathways in veins and the aorta have recently attracted a lot of attention from researchers. *"IL-18"* is an example of a cytokine that promotes inflammation and is mostly produced by "monocytes and macrophages". It exhibits significant effects on macrophages as well as T cells (Z. Chen et al., 2020) which are two distinctive kinds of cells implicated in the development and consequences of "inflammatory plague", which ultimately contribute to "cardiovascular disease". When it comes to the production of "IFN-g, IL-18" has the strongest synergism with *"IL-12*." these later "cytokines" are expressed in serum as well as in the blood cells of peripheral organs in animal models of atherosclerosis (Spaccamela, 2019; Chen, 2020).The immunoinflammatory response is what determines the atherosclerotic lesion's size and content, and several cytokines have been shown to play an essential role in this

process.(Xu, 2021; Soleimani, 2021) According to Vallejo; (2019); Helmke, (2019) IFN-g has a significant impact on the amount of collagen that is found in atherosclerotic plaques. In part due to the fact that it inhibits the body's natural production of collagen.

CHAPTER VI:

Conclusion and Recommendations

5.1. Conclusion

"Macrophages" are principally responsible for the synthesis of *IL-eighteen,* despite the fact that "hepatocytes and keratinocytes" are also capable of producing it. Inhibiting a co-factor that is essential for the production of "Th1 cells" is the major goal of this strategy. It does this by increasing the activity of "NK cells" as well as stimulating the production of interferon gamma. Cytokines are proteins that are created in reaction to infections and other antigens. Cytokines are accountable for the regulation and mediation of immune and inflammatory responses. Cytokines are produced in response to infections and other antigens.

The production of *interleukin* is a process that has its own built-in restrictions on how much can be produced. Due to the instability of messenger RNA, which is responsible for coding for the vast majority of *interleukins*, a transient synthesis takes place. These molecules are promptly eliminated from the body after they have been created, as soon as the process is complete. During the process of cellular responses to *interleukins,* which involve both up- and down-regulatory processes, the expression of genes that code for inhibitors of cytokine receptors is both induced and taken part in by the cell. These genes' products are cytokine receptors.

The findings of this research report indicated that the level of *IL-18* expression was found to be higher in the tissue cells of the veins, while the level of expression was found to be lower in the tissue cells of the aorta.

When the expression levels of "interleukin" eight and "interleukin" eighteen are compared, it can be seen that IL-eighteen levels are significantly higher, particularly in the control group, as compared to IL-eight.

Additionally, it can be seen that IL-8 levels are significantly reduced in veins tissues for both the control group and the patient group. The venous tissue cells of the control group demonstrated higher levels of "interleukin 8 and 18 expression" in contrast with the patients group. There is a possibility that the downregulation of "interleukins 18" in the patient group is the underlying cause of a particular health issue. This lends credence to the idea that the generation and overexpression of interleukins in the

control group conferred some degree of protection for the control group compared to the afflicted groups. On the other side, there is evidence that links high expression of interleukins to major health issues.

It is possible that vein tissue can be relevant for the study of "cytokines" due to the fact that "IL-18 and IL-8" are more significantly expressed in veins than in the aorta.

5.2. POLICY RECOMMENDATIONS

The most important behavioural risk factors of heart disease and stroke are unhealthy diet, physical inactivity, tobacco use and harmful use of alcohol. The effects of behavioural risk factors may show up in individuals as raised blood pressure, raised blood glucose, raised blood lipids, and overweight and obesity**.** These “intermediate risks factors” can be measured in primary care facilities and indicate an increased risk of heart attack, stroke, heart failure and other complications.

Cessation of tobacco use, reduction of salt in the diet, eating more fruit and vegetables, regular physical activity and avoiding harmful use of alcohol have been shown to reduce the risk of inflammation and cardiovascular disease.

5.3. RECOMMENDATİONS ACCORDİNG TO THE FİNDİNGS

It is critical to identify those at highest risk of CVDs and ensuring they receive appropriate treatment can prevent premature deaths.

It is important to include interleukin 8 and 18 screening in the health system especially for the elderly, pregnant women and children, since the expression of these protein are physiologically important for the control of inflammation and CVDs.

5.4. RECOMMENDATİONS FOR FURTHER RESEARCH

More and research is needed to increase our understanding of the disease to uncover critical regulatory mechanisms. This could serve as a possibility that fresh “therapy” alternatives could be developed in the future, which will assist in lowering the mortality rate that is linked with the ailment.

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Appendix A

Ethical Approval Document

NEAR EAST UNIVERSITY
SCIENTIFIC RESEARCH ETHICS COMMITTEE

RESEARCH PROJECT EVALUATION REPORT

Meeting date :27.04.2023
Meeting Number :2023/113
Project number :1706

The project entitled **"The gene expression profile pathways of Interleukin 8 and Interleukin 18 genes in veins and aorta obtained from patients with various cardiovascular disorders"** (Project no: NEU/2023/113-1706), which will be conducted by Doç. Dr. Mahmut Ergören has been reviewed and approved by the Near East University Scientific Research Ethical Committee.

Prof. Dr. Şanda Çalı
Near East University
Head of Scientific Research Ethics Committee

Committee Member	*Decision*	*Meeting Attendance*
	Approved (✓) / Rejected (X)	*Attended (✓) / Not attended(X)*
Prof. Dr. Tamer Yılmaz	✓	✓
Prof. Dr. Şahan Saygı	✓	✓
Prof. Dr. İlker Etikan	✓	✓
Assoc. Prof. Dr. Mehtap Tınazlı	✓	✓
Assoc. Prof. Dr. Nilüfer Galip Çelik	X	X
Assoc. Prof. Dr. Dilek Sarpkaya	✓	✓
Assoc. Prof. Dr. Gulifeiya Abuduxike	✓	✓
Assoc. Prof. Dr. Burçin Şanlıdağ	✓	✓

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Appendix B

Turnitin Similarity Report

THESIS

ORIGINALITY REPORT

11%	8%	8%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	docs.neu.edu.tr Internet Source	2%
2	www.nature.com Internet Source	1%
3	www.mdpi.com Internet Source	1%
4	Abdulhamied Alfaddagh, Seth S. Martin, Thorsten M. Leucker, Erin D. Michos et al. "Inflammation and cardiovascular disease: From mechanisms to therapeutics", American Journal of Preventive Cardiology, 2020 Publication	1%
5	molmed.biomedcentral.com Internet Source	1%
6	en.wikipedia.org Internet Source	1%
7	Simon Keckstein, Sophia Pritz, Niklas Amann, Sarah Meister et al. "Sex Specific Expression of Interleukin 7, 8 and 15 in Placentas of Women with Gestational Diabetes", International Journal of Molecular Sciences, 2020 Publication	1%
8	orca.cf.ac.uk Internet Source	<1%
9	www.science.gov Internet Source	<1%
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11	Cytokine Frontiers, 2014. Publication	<1%
12	inflammregen.biomedcentral.com Internet Source	<1%
13	"INTERLEUKIN-18 REGULATES BOTH TH1 AND TH2 RESPONSES", Annual Review of Immunology, 04/2001 Publication	<1%
14	Weidlich, Simon, Ana-Maria Bulau, Tobias Schwerd, Johanna Althans, Roland Kappler, Sibylle Koletzko, Doris Mayr, and Philip Bufler. "Intestinal Expression of the Anti-inflammatory Interleukin-1 Homologue IL-37 in Pediatric Inflammatory Bowel Disease :", Journal of Pediatric Gastroenterology and Nutrition, 2014. Publication	<1%
15	Dong-Ik Kim, Hyun-Seon Eo, Jin-Hyun Joh. "Identification of differentially expressed genes in primary varicose veins", Journal of Surgical Research, 2005 Publication	<1%
16	Masaki Shimizu, Syuji Takei, Masaaki Mori, Akihiro Yachie. "Pathogenic roles and diagnostic utility of interleukin-18 in autoinflammatory diseases", Frontiers in Immunology, 2022 Publication	<1%
17	Seppe Cambier, Mieke Gouwy, Paul Proost. "The chemokines CXCL8 and CXCL12: molecular and functional properties, role in	<1%