The Role of Some Hormone Levels and Histopathological Changes in Thyroid Gland and Testes in Experimentally Induced Myocardial Infraction in Male Rats

Noori Mohammed Luaibi

Department of Biology, College of Science, AL-Mustansyria University, Baghdad-Iraq.

Abstract

Acute myocardial infarction is a common cardiac emergency, with the potential for substantial morbidity and mortality. Myocardial infarction occurs when myocardial ischemia, a diminished blood supply to the heart, exceeds a critical threshold and overwhelms myocardial cellular repair mechanisms designed to maintain normal operating function and homeostasis. The aim of the current study was to evaluate the alterations in hormone concentrations TSH, T3, T4, and testosterone in experimental rats has been induced for myocardial infarction. According to statistical analysis revealed that the levels of T3, T4 and testosterone were decreased in comparison with healthy control group and the decreasing value of these hormones were continuing to occur especially in 7 and 14 days after myocardial infarction occurs. While the levels of TSH illustrated increment in various periods of time used in this study after admission of myocardial infarction, in addition to histological changes that had occurred in thyroid and testes sections. Thyroid sections showed follicles empty from colloid and degenerative changes in thyroid follicular cells in long period of time after acute myocardial infarction, while testes showed immaturity of spermatogonial cells with deformed sperms inside the lumen.

Keywords: Myocardial infarction, TSH, T3, T4, Testosterone.

Introduction

Acute myocardial infarction is a common cardiac emergency, with the potential for substantial morbidity and mortality¹. Myocardial infarction occurs when myocardial ischemia, a diminished blood supply to the heart, exceeds a critical threshold and overwhelms myocardial cellular repair mechanisms designed to maintain normal operating function and homeostasis².

Over the past several years, great achievement has been made in the management of cardiovascular diseases that depended on using experimental animal models which has out lined the pathogenesis, mechanisms and progress underlying cardiovascular diseases at the cellular and molecular level³. This has allowed the development of many effective treatment strategies⁴. Most myocardial infarction are caused by a disruption in the vascular endothelium associated with an unstable atherosclerosis plaque that stimulates the formation of an intracoronary thrombus, which results in coronary artery blood flow occlusion, if such an occlusion persists for more than 20 minutes, irreversible myocardial cell damage and cell death will occur⁵. The usual initiating mechanism for acute myocardial infarction is rupture or erosion of a vulnerable, lipid-laden, atherosclerotic coronary plaque, resulting in exposure of circulating blood to highly thrombogenic core and matrix materials in the plaque⁶. Disruption of the endothelial surface can cause the formation of thrombus via platelet-mediated activation of the coagulation cascade, if a thrombus is large enough to occlude coronary blood flow, an M.I can result, the severity of an M.I depends on three factors: the level of the occlusion in the coronary artery, the length of time of the occlusion and the presence or absence of collateral circulation⁷.

The path of physiological alterations that transpire during M.I occur in two stages: early changes at the time of acute infarction and late changes during myocardial healing and remodeling.
Material and Methods

Experimental Study: Laboratory Animals

In the present study, 35 adult male rats, weighting 250-300g were used for induced myocardial infarction by cryo-injury method. Animals were divided into three groups each group contains 5 rats in case of acute and chronic M.I and sacrificed after (4/hr., 8/hr., 24/hr.) (7/day, 14/day, 28/day) respectively, all groups compared with 5 male adult rats healthy weighing 250-300g as a control group. Animals were purchased from National Center for Drug Control and Research (NCDCR), Ministry of Health and housed in the animal house of the College of Science/ Al-Mustansiriyah University. They were kept in standard plastic cages with a metal network cover, under climate controlled conditions of the animal house with temperature 25±2ºC and 10:14 light and dark cycle. Rats were provided with water and food ad libitum.

Myocardial Infarction Model

Myocardial infarction was induced following a standardized protocol, 30 adult male rats weighting 250-300 g, were anesthetized with diethyl ether. Under aseptic condition, the rat placed a supine position in a temperature-control plate (37 C0). Shaving the chest and sterilized by antiseptic solution (Alcohol 70 %), the rat heart was exposed through a 1.5 cm left lateral thoractomy incision. Cryo–injury was produced with an aluminum or metal probe (0.5 cm in diameter) cooled to – 190 ºC by immersion in liquid nitrogen and was applied to left ventricular (L.V) free wall for 15 seconds, after 5 second rest, this procedure was repeated two times and infarct area was visualized.

The muscle layer and skin incision were closed with 5-0 and 3-0 silk suture respectively and the animals were returned to their cages and carefully monitored for 4 hours after operation, dressing the incision by using fucidin antibiotic cream in addition to benzathin pencillin G (1500u/ml) and procaine pencillin G (1500u/ml) were given intra- muscularly (0.4 ml per rat) after each operation twice a day for the first 48 hours.

Animals were divided into two experimental groups:

- First groups: Acute MI (4/hr., 8/hr., 24/hr.) (15 rats).
- Second groups: Chronic MI (7/day, 14/day, 28/day) (15 rats).

Collection of Blood Samples and Organs

From each rat (acute M.I groups, chronic M.I groups and control groups). The end of each experiment animals were fully anaesthetized by diethyl ether for several minutes and blood samples were obtained by heart puncture. 3 ml of blood collected from each rat was used to obtain sera separated by centrifugation 3000 rpm for 10 min, then they were kept in -20ºC until use. The animals were dissected and their organs, thyroid gland and testes were excised then fixed with 10 % formalin.

Hormone Kits

Measurement of the Levels of Hormones Concentration

It was represented by the enzyme immunoassay test (ELIZA) for the quantitative determination of concentrations of thyroid gland hormones T3 according to2, T4 according to3and TSH according to1. Also, the reproductive hormone testosterone was measured according to12.

Histopathological Preparation

The Preparation for histological sections was performed according to the method of13.

Results and Discussion

Thyroid Functions and Histological Sections

Figures 1 to 3 illustrate the levels of various hormones in male rats experimentally induced myocardial infarction.

According to statistical analysis the levels of T3 and T4 were decreased in comparison with healthy control group. Decrement in the level of these hormones were continuous in the serious period times especially in 7 and 14 days after myocardial infarction occurs, while TSH levels showed highly significant increase (p<0.01) between various period times after attack.

TSH Levels

Statistical analysis of the present study of the effect of myocardial infarction on thyroid
function that included TSH level in Figure (1) shows: Highly significant increase (p<0.01) in TSH levels in rat groups with myocardial infarction in the different periods of time from 7 days to 28 days (0.394±0.022), (0.422±0.022), (0.484±0.021) (μU/ml), respectively, compared to control groups (0.232±0.024)(μU/ml).

T3 levels
Statistical analysis of the present study of the effect of myocardial infarction on thyroid function that included T3 level in Figure (2) shows: Highly significant decrease (p<0.01) in T3 levels in rat groups with myocardial infarction in the different periods of time from 8 hrs to 28 days (1.342±0.005), (1.266±0.003), (1.225±0.020), (1.025±0.002), (0.877±0.018), (0.656±0.019), (0.471±0.029) (ng/ml), respectively, compared to control groups (1.497±0.022)(ng/ml).

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T4 levels

Statistical analysis of the present study of the effect of myocardial infarction on thyroid function that included T4 level in Figure (3) showed: Highly significant decrease (p<0.01) in T4 levels in patient groups with myocardial infarction in the different periods of time from 2hrs to 28 days (4.852±0.019), (4.800±0.021), (4.721±0.035), (4.400±0.018), (3.862±0.023), (3.222±0.030), (2.851±0.022), (2.287±0.023)(µg/dl) respectively, compared to control group (4.961±0.034)(µg/dl).

Histological Sections of Thyroid

The main histological changes in thyroid tissues in all experimentally induced myocardial infarcted rats in different periods of time compared to control groups are shown as follows:

Thyroid sections showed different histological changes after 24 hours showing: Normal structure of follicles, presence of colloid material inside the follicles with few follicles empty from colloid, figure(5). While in experimental groups after 1 week from myocardial infarction sections showed scalloping of colloid material at the apical pole of follicular epithelial cells with some follicles empty from colloid as shown in figure(6). After 2 weeks from myocardial infarction sections observed more follicles empty from colloid, figure(7). But the worst effects demonstrated in the experimental group after 4 weeks from myocardial infarction induction, sections showed inactive follicles containing no colloid material with degenerative changes of thyroid follicular cells as in figure (8) in comparable with the control group, figure (4).

Figure 4: Section of thyroid gland from the control group showing normal structure appearance of: 1. Thyroid follicles of different size and shape filled with 2. Colloid materials (H&E) 100x.

Figure 5: Section of thyroid gland from experimental groups after 24 hours showing: 1. Normal structure of follicles 2. Presence of colloid material inside follicles 3. Few follicles empty from colloid. (H&E) 100x.
Thyroid dysfunction is associated with various levels of cardiovascular risk, as observed by independent changes in the severity of risk factors that have been associated with cardiovascular morbidity in different studies. Subclinical hypothyroidism is defined as a serum thyroid-stimulating hormone (TSH) level above the upper limit or mild thyroid failure that is a common problem, with a prevalence of 3% to 8% in the population without known thyroid disease. Subclinical hypothyroidism (SCH) after admission for an acute cardiac problem has been linked to an increase in cardiac mortality and overall death, even mild hypothyroidism, known as subclinical hypothyroidism, is associated with worse cardiovascular outcomes. In a study by results showed that hypothyroidism has been found to be associated with cardiovascular disease, subclinical hypothyroidism is highly prevalent and is associated with a greater frequency of aortic atherosclerosis and myocardial infarction. Some studies have reported an association between thyroid autoimmunity and coronary heart disease. But other studies have not. Another study showed down regulation of the thyroid hormone system can occur after cardiac diseases, this condition, which has been called 'low T3 syndrome', is
characterized by a change in thyroid homeostasis which occurs as a result of impairment in the normal feedback response due to low T3 levels and disruption in conversion of precursor hormone T4 to T3, the decrease in active hormone T3 leads to further impairment in cardiac functions.

A hypothesis have been addressed that low T3 state after acute MI may contribute to the changes in cardiac specific gene expression and further compromise the cardiac myocyte in its response to the ischemic injury, serum total T3 and T4 levels were measured in an animal model during the 4 week period after coronary artery ligation, serum T3 levels fell within 1 week of the acute infarct, and remained 40% lower than control, while serum T4 levels were unchanged after acute MI and throughout the 4 week period after coronary artery ligation.

In severe illness of any cause, down regulation of the thyroid hormone system may occur, the thyroid hormone system is rapidly down regulated in AMI and level of T3 reduce is proportional to the severity of cardiac damage. A close relationship exists between the thyroid gland and the heart beginning in development and extending to adult physiology, the important physiological link is affirmed by the predictable changes in cardiovascular function that occur across the entire range of thyroid disease states, hypothyroidism results in impaired left ventricular (LV) contractile and relaxation functions, increased systemic vascular resistance, and low cardiac output, similar to that seen with congestive heart failure. There are numerous parallels between heart failure and hypothyroidism, including rise to low serum thyroid hormone, especially T3 levels and a variety of other cardiac disease states. Clinical hypothyroidism is associated with premature atherosclerosis and raised prevalence of coronary heart disease which is partially due to the lipid abnormalities that are often found in hypothyroidism.

Following experimental coronary artery ligation in an animal model of AMI, heart failure was linked to reduced thyroid hormone receptor expression in the myocardium, resulting in tissue hypothyroidism. From a study by data regarding the association between subclinical hypothyroidism and cardiovascular disease outcomes, observed that subclinical hypothyroidism is associated with an increased risk of coronary heart disease CHD events and CHD mortality in those with higher TSH levels, especially in those with a TSH concentration of 10 mIU/L or greater. Acute myocardial infarction (AMI) and its related complications act for a major public health problem that can cause morbidity and mortality.

Testis Functions and Histological Sections

Testosterone Levels

Statistical analysis of the present study of the effect of myocardial infarction on testes functions that included Testosterone level in figure (9) shows: Highly significant decrease (p<0.01) in testosterone levels in patient groups with myocardial infarction in different periods of time from 8 hrs to 28 days compared to control groups.

![Figure 9](image)

Figure 9: Different concentration of Testosterone levels in male rats experimentally induced myocardial infarction in different periods of time in comparison with control group. (***) highly significant decrease (P<0.01).
Histological Changes of Testes

The main histological changes in all experimentally induced myocardial infarcted rats in different periods of time compared to control groups is shown as follows: Testes sections showed no histological changes in experimental groups after 24 hours from myocardial infarction showing nearly normal appearance of seminiferous tubules with presence of sperms inside the lumen, figure (11). Experimental groups after 1 week from myocardial infarction showed sections of testis with rare seminiferous tubules observed immaturity of spermatogenesis, figure (12).

Sections of testis from the experimental groups after 2 weeks showed certain seminiferous tubules with immaturity of spermatogonial cells, presence of deformed sperms inside the lumen while other tubules contain normal looking sperms, figure (13 A & B). But Section of testis from the experimental group after 4 weeks showed majority of seminiferous tubules with immaturity of spermatogonial cells, no sperms inside the lumen with few tubules showing mature spermatogonia and presence of sperms, figure (14), in comparable with control section in figure (10).

Figure 10: Section of testis from the control group showing: 1. Elongated and rounded seminiferous tubules with normal structure appearance 2. Full maturation of spermatogonial cells 3. Presence of sperms inside the lumen. (H&E) 200x.

Figure 11: Section of testis from the experimental group after 24 hours showing: 1. seminiferous tubules with nearly normal appearance 2. Presence of sperms inside the lumen 3. Spermatogonial cells.(H&E) 200x.

Figure 12: Section of testis from the experimental group after 1 week showing: 1. Majority of seminiferous tubules showing normal maturation of spermatogonial cells with presence of sperms inside the lumen 2. Rare tubules showing immaturity of spermatogenesis. (H&E) 200x.
Cardiovascular diseases are still the highest resultant of death worldwide, there are several risk factors that are linked to cardiovascular diseases, they include smoking, hyperlipidemia, and gender among others, for sex hormones, especially the androgen and its receptor, androgen receptor (AR), have been linked to many diseases with gender difference. However, investigated sex hormonal levels in men with chronic heart failure, they found that they were 28% deficient of testosterone, and less circulating testosterone levels in the body has been related to exercise capability in male patients with chronic heart failure. The reduction in both serum and total testosterone with age have been linked to several disease states in men. Especially, ischemic heart disease and cardiac failure have been linked to this natural biochemical decline in testosterone.
Evidence from 34 suggest that low is more than high, testosterone (T) is associated with increased male morbidity and mortality, as low T particularly is associated with increased risk of cardiovascular (CV) death and it is possible that both cardiovascular disease CVD and low T are associated with another.

Testosterone levels in men slowly reduces with aging and reduces more abruptly due to medical illness or medications such as myocardial infarction, decrement in T level may also be associated with mortality because low testosterone levels may be a marker for underlying medical morbidity, the evidence that supports this hypothesis is that reduction in testosterone level is associated with both acute and chronic medical conditions35. In a study by 36 it has been reported that androgens had a curvilinear relationship with mortality and CV events, as both low and high levels were associated with an increased risk. The most prevalent type of specific mortality associated with decline in testosterone level is cardiovascular (CV) mortality, which includes mortality due to coronary heart disease, myocardial infarction or heart failure37.

In a study on 30 male patients that had acute myocardial infarction, results observed that attack of acute myocardial infarction in men can alter the sex hormone levels, whether, the hormonal change show enhanced aromatization of testosterone to estradiol or were due to some other unknown mechanism38.

39 Has reported that thyroid hormone deficiency affects all the tissues of the body, which includes multiple endocrine changes that alter growth hormone, glucocorticoids, corticotrophin, and gonadal function, also primary hypothyroidism is associated with hypogonadotropic hypogonadism, so men with primary hypothyroidism observe reduced free testosterone concentrations, as thyroid hormone is known to affect sex hormone-binding hormonal globulin (SHBG) concentrations. In consistent alterations in gonadal steroid genesis and pituitary functions have been reported in hypothyroid males, hypothyroidism was found to be associated with an increase in the circulating level of total cholesterol and a reduction in the levels of progesterone and testosterone, without any change in the serum levels of estradiol and gonadotrophins, the decline in serum testosterone level could be explained by decrease in serum triiodothyronine , a higher rate of conversion of testosterone to estradiol or the further decline in the rate of conversion of progesterone to testosterone40. Two studies and others have noted that hypothyroid men tend to have lower free testosterone concentrations, GnRH is produced by the hypothalamus that triggers LH, in turn LH stimulates the testes to produce testosterone, which means that hypothyroidism sabotages the first step in the testosterone production assembly line39. TSH is a signaling hormone synthesized and released from pituitary gland that signals the thyroid to start producing more thyroid hormones, in some kinds of hypothyroidism, TSH starts to rise, thyroid hormones are similar to testosterone and estradiol in the sense that they have two forms bound and unbound, so low free T3 and/or free T4 values indicate hypothyroidism, which makes sense showing a low output of hormones from the thyroid41. Myxedema a type of hypothyroidism in men is thought to cause infertility and impotence, in eight consecutive men with primary hypothyroidism testicular function was investigated, results showed decline in serum testosterone and testosterone /estradiol-binding globulin concentrations in four of the patients, it is concluded that abnormalities of gonadal function are common in men with primary hypothyroidism12. A study was undertaken to study the effect of neonatal onset hypothyroidism on Leydig, plasma and testicular interstitial fluid (TIF) sex steroid concentration at different age groups of Wistar rats ,leydig cell number in hypothyroid rats were less than the age-matched control and the diameter of Leydig cells in hypothyroid rats was smaller than the controls, hypothyroid rats had Plasma testosterone, DHT and estradiol were decreased in all hypothyroid rats, TIF testosterone and progesterone titer showed a consistent decrease in hypothyroid rats irrespective of the duration, data indicate that neonatal onset hypothyroidism adversely affected Leydig cell proliferation along with impaired steroidogenesis43. Sertoli cells are rich in thyroid hormone receptors in neonatal testes, therefore their important effects of hypothyroidism in this tissue44.
References


