CHAPTER 6

SUMMARY AND CONCLUSION

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Heavy metal contamination in marine ecosystems is of global concern. Metals generally enter the aquatic environment via atmospheric deposition, geological matrix erosion or due to anthropogenic impacts caused by industrial effluents, domestic sewage, mining wastes, and agricultural activities. It will be toxic to physiological and behavioral effects on the aquatic biota which results in adverse effects on humans.

The contamination levels of the aquatic environment by heavy metals can be estimated by analyzing water, sediments and marine organisms. The levels of heavy metals in mollusks and other invertebrates are often considerably higher than in other constituents of marine environment due to their habitat and their feeding habits. Compared to sediments, mollusks exhibit greater spatial sensitivity and therefore, are the most reliable tool for identifying sources of biologically available heavy metal contamination.

In this aspect mussels are used as test organisms for biomarker survey because they are widely distributed geographically, sessile and resistant to a wide range of metal concentrations. Among the most used biomarker for pollution in marine environment, metallothioneins have been particularly useful as monitoring device, namely as a contaminant specific biochemical indicator of metal exposure. Therefore, the primary purpose of the present study was to obtain quantitative estimation of metallothionein concentrations in mussels as a biomarker of exposure to heavy metals, to monitoring the pollution of Abu Qir bay (El-Maadiya region).

The present results indicated that the studied area was contaminated with some heavy metals as cadmium, lead, copper, chromium, and zinc. Meanwhile, the present study proved the presence of measurable amounts of metallothionein in mussels collected from the studied area.

Living around polluted areas is one of the most common sources of exposure to environmental toxicants. Of these toxicants, heavy metals are widely used in foundries, mining, and manufacturing industries. Once heavy metals accumulate in the ecosystem components; such as air, soil, and water the risk of human exposure increases among industrial workers, as well as, the people who live near polluted areas.

Abu Qir Bay is a shallow semi-circular tideless basin east of Alexandria. The bay is adjacent to one of the most populous, most industrialized and most commercialized coastal metropolitan areas in Egypt. Residents of El Maadiya region face immediate environmental impact of heavy metals pollution. Therefore, the second purpose of the present study was conducted to study the risk assessment of the environmental pollution in Abu Qir bay on human health through the determination of some metals in blood of all studied subjects, metallothionein and the detection of oxidative stress through the estimation of malondialdehyde (MDA), glutathione content (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT), and their impact on the gene expression of insulin-like growth factor 2 (IGF-2).

The present study was conducted on 50 subjects, was divided to two groups: group I contain 10 control subjects and group II comprised of 40 fishermen.

The results of the current study proved the presence of high concentrations of some heavy metals as cadmium, chromium, lead, copper and Zinc in the blood of fishermen group, associated with striking significant high levels of metallothionein in their erythrocytes.

Metallothionein (MT) is thought to be involved in homeostasis of the essential metals, copper, and zinc, as it is the major zinc and copper binding protein in many tissues, and there is a close relationship between tissue MT and zinc content.

MT may be acting as a sensor of the localized intracellular redox balance and may itself influence redox balance through GSH and the known antioxidant properties of zinc. Over expression of metallothionein reduces the sensitivity of cells and tissues to free radical damage and metallothionein genes are transcriptionally activated in cells and tissues in response to oxidative stress.

Metals are small entities when compared to organic materials and their reactions with living matter, are seemingly simple to evaluate. However, the picture emerging today shows a very complex pattern of metal interactions with cellular macromolecules, metabolic and signal transduction pathways and genetic processes. A special feature of metal biology is the fact that even metals that are essential for the sustainment of life (such as iron and copper) may become toxic depending on the oxidation state, complex form, dose and mode of exposure.

Therefore, the current results elucidated that fishermen group exposed to various types of heavy metals which evident by the presence of cadmium, chromium, copper, lead and zinc in their blood which generated a sever oxidative stress which manifested by the presence of highly levels of malondialdehyde, accompanied by severe decrease in the antioxidant defense in their blood i.e. decrease in the glutathione content and decrease in the antioxidant enzymes activities of glutathione peroxidase and catalase.

The induction of oxidative stress is an attractive hypothesis to explain mutagenic and carcinogenic effects of metals. They have been shown to induce the formation of reactive oxygen and nitrogen species in vivo and in vitro in mammalian cells. Frequently the formation of hydroxyl radicals, most probably by Fenton-and Haber–Weiss-type reactions, has been detected. These radicals are known to cause oxidative damage to lipids, proteins and DNA.

Besides generating DNA damage directly, reactive oxygen species at low concentrations function as mitogenic signals and activate redox-sensitive transcription factors. Hence, oxidative stress may not only initiate tumor development by mutagenesis but also deregulate cell growth and promote tumor growth depending on extent and time of interference.

Metals modulate gene expression by interfering with signal transduction pathways that play important roles in cell growth and development. The underlying mechanism involves formation of the superoxide radical, hydroxyl radical, and finally the production of mutagenic and carcinogenic malondialdehyde, 4-hydroxynonenal, and exocyclic DNA adducts. Carcinogenic metals and metalloids such as As, Cd, Ni and Co can also inhibit zinc finger-containing DNA repair proteins.

In our laboratory, Aziza et al (2010) found that pollution of Abo Qir bay (El Maadiya region) with aromatic amines induced a panic oxidative stress and high frequencies of chromosomal aberrations in the peripheral blood of fishermen. In addition, the results of the

present study revealed that there was a high significant increase in the gene expression of Insulin-like growth factor-2 (IGF-2) in the blood of the aforementioned fishermen group.

Insulin-like growth factor-2 (IGF-2) is involved in the regulation of liver cell growth and metabolism. IGF-2 is structurally related to proinsulin, IGF-1, and relaxin. The mitogenic and antiapoptotic properties of both IGF peptides as well as differentiation-related signaling are mediated primarily through IGF-1 receptor (IGF-1R). IGF-2 is physiologically expressed at high levels in various human and rodent fetal tissues such as liver, kidney, and skeletal muscle. In contrast, it is down regulated or virtually absent in the corresponding adult organs.

In addition, circulating IGF-2 arises in part from liver, its concentration having been reported to reflect hepatic integrity. Liver disease could, therefore, confound interpretation of the concentration. Gene expression and plasma protein signatures may enable early diagnosis of cancer in the future.

In a study of Baddour et al (2011), a significant positive correlation was observed between IGF-2 expression and the grade of inflammatory activity, this is in accordance with the findings of Grisham et al (2001) that; upregulation of IGF-2 in chronic hepatitis results from the combined actions by cytokines produced by chronic inflammatory cells that infiltrate damaged livers and viral transactivation. In this respect, from the findings of the present study it may be suggested that the investigated fishermen group may suffered from insidiously hepatitis with a consequential serious effect on fishermen health.

Recent research findings suggest much potential clinical utility for IGF-2 testing in the context of liver cancer. The possibility of predicting hepatocarcinogenesis by genetic testing is perhaps the most exciting. Genomic assays that provide molecular signatures for multiple genes, including IGF-2, may also predict cancer risk.

In some instances, increased IGF-2 gene expression *(i)* has been correlated with increased rates of cell mitotic activity, as estimated by proliferating cell nuclear antigen (PCNA) expression and *(ii)* may contribute to tumoural angiogenesis.

Similarly, Igf-2 gene expression was reactivated during hepatocarcinogenesis in transgenic mice and was associated with high replicative activity, but not with changes in apoptosis. Interestingly, re-expression and overexpression of the Igf-2 gene in mouse and human HCCs, respectively, was concomitant *(i)* with the re-activation of a fetal pattern of gene expression, and *(ii)* with silencing of the liver-specific promoter P1 in human HCCs. In addition, overexpression of the IGF-2 gene in human preneoplastic foci and HCCs has also been reported to be associated with the restoration of an allelic imbalance at the IGF-2 locus. That overexpression of IGF-2 may be involved in the hepatocarcinogenetic process, or in HCC cell proliferation could be deduced from ex vivo experiments.

Finally in some cases, accumulation of IGF-2 in HCCs tissue could be due to up-regulation of IGF-2 gene transcription by p53mt249, a gain-of-function mutant of p53 frequently observed in patients that have developed HCCs after prolonged exposure to aflatoxin B1. p53mt249 enhances transcription from the fetal IGF-2 promoter P4.

Tumor development is characterized by a deregulation of cell growth and differentiation. Carcinogenic metal compounds may alter cell growth by several distinct mechanisms, either affecting the expression of growth stimulating factors or inactivating growth control mechanisms. With respect to the former, some metal ions are found to activate mitogenic

signaling pathways and induce the expression of cellular proto-oncogenes. Furthermore, epigenetic mechanisms, such as hypo- or hyper-methylation of DNA or disturbed histone acetylation, may contribute to modified patterns of gene expression. Changes in gene regulation are observed prior to manifestation of tumors. Initially, they are not fixed by mutation, and the agent must be present for an extended time period to cause persistent modifications, which can be genetically fixed during tumor development. Concerning the interference with cellular growth control, some metal carcinogens have been shown to inactivate the tumor suppressor proteins p53 and/or down regulate the expression of tumor suppressor genes Fhit, p16, p53 and of senescence genes. Finally, metal ions may deregulate cell proliferation by inactivating apoptotic processes resulting in adaptation to the cytotoxicity of the metal.

There is increasing evidence for interaction between IGF-2 and p53 in cancer development. Normally, IGF-2 transcription is repressed by the tumor suppressor p53, which also increases IGFBP3 and suppresses IGF1R expression. Decreased activity of p53 in tumors, therefore, increases both IGF-2 expression and action. Recent data suggest that increased IGF-2 signaling favors tumor development by suppressing activity of the p53 pathway.

The aforementioned finding represented a good interpretation to the herein results which elucidated that severe oxidative stress in the blood of fishermen group predisposed in up-regulation of IGF-2 gene by hypomethylation of DNA.

Conclusively, the present study elucidated that El Maadiya region is polluted with heavy metals, at the same time another two studies in the same laboratory proved the pollution of El Maadyia region with some aromatic amines and some polycyclic aromatic hydrocarbons. The pollution induces a panic oxidative stress in fishermen in the vicinity of this area. The risk will persist because the high increase levels of malondialdehyde coincide with high decrease in the levels of the antioxidant glutathione, and the enzymatic activities of catalase and glutathione peroxidase. It was proved that carcinogenic metal compounds often are comutagenic, that is, they enhance the mutagenicity of other genotoxic agent. Indeed, many carcinogenic metal compounds at low concentration have been identified as inhibitors of the repair of DNA damage that is caused either by other xenobiotics or by endogenous factors. Inhibition of repair and persistent DNA damage results in genomic instability which may become especially deleterious under conditions of acceleration cell proliferation and/or impair apoptosis. The present results exhibited the presence of high significant level of metallothionein in fishermen blood and there is a positive correlation between metallothionein and malondialdehyde, while there are negative correlation between metallothionein and the antioxidant glutathione, glutathione peroxidase, and catalase, the present finding is in agreement with other studies which elucidated that increasing the level of ROS and oxidative stress induce increase expression of MT mRNA and protein levels, which can increase tumor cell survival and viability due to their antioxidative and antiapoptotic effects.

Meanwhile, the present data emphasized that there was a high significant increase in the gene expression of IGF-2, and there is a positive correlation between expression of IGF-2 and MT. In addition a negative correlation between gene expression of IGF-2 and the level of GSH as well the enzymatic activities of GPx and CAT, furthermore, Aziza et al (2010) found high frequencies of chromosomal aberrations in lymphocytes of peripheral blood of the same group of fishermen in the same area.

Oxidative stress mechanisms generated by xenobiotics may also involve aberrant epigenetic modification of DNA and histones via the depletion of glutathione and changing the ratio of reduced GSH and its oxidized form, GSSG. Oxidative stress may also alter epigenetic modification via mitochondrial dysfunction. To be inhibitors, isoflavones, polyphenol, zinc and cadmium may inhibit DNA methyl transferases (DNMTs) directly and indirectly and further inhibit methylation of candidate genes.

Up-regulation of IGF-2 in some hepatocytes may lead to high focal IGF-2 levels sufficient to saturate local IGF-2 binding capacities, and may result in an increased susceptibility to cellular dedifferentiation and, ultimately, liver cancer. Down regulation of hepatocellular M6P/IGF-2R and upregulation of IGF-2 seem to be early events in hepatocarcinogenesis prior to the appearance of morphologically distinct dysplastic lesions. Elevated focal IGF-2 transcript levels may therefore indicate an increased risk for hepatocellular and cholangiocellular carcinomas.

In addition, circulating IGF2 arises in part from liver, its concentration having been reported to reflect hepatic integrity. Liver disease could, therefore, confound interpretation of the concentration. Gene expression and plasma protein signatures may enable early diagnosis of cancer in the future.

Then, the coexistence of urinary metabolites of aromatic amines and polycyclic aromatic hydrocarbons with heavy metals in the blood of fishermen group a long with the coincidence of oxidative stress concomitant with increase metallothionein levels, chromosomal aberrations, and overexpression of IGF-2 gene let the fishermen of El Maadiya region are under high risk to cancer.

RECOMMENDATION

RECOMMENDATION

1. Prevention of drainage of factories waste products in lakes or in the sea, and the necessity of treating the wastes before drainage.

2. Importance of clinical follows up by laboratory and radiological investigations once every six months at least to insure absence of any disease or tumors.

3. A challenge for the future will be to understand how IGF2 interacts with other components of the system at tissue level to influence cancer development and progression. Similarly, genetic and epigenetic changes affecting IGF2 need to be considered in the context of the whole genome. While there has been abundant research into the disease association of IGF system components, future work needs to place a greater emphasis on the clinical value of measurement of these components, including IGF2, as diagnostic tests.

4. Applications of the aforementioned studied parameters "Blood glutathione contents, erythrocytes catalase activity, erythrocytes glutathione peroxidase, malondialdehyde and metallothionein" for the early detection of human health risk resulting from the exposure to environmental pollutants.

5. The area of study required more environmental monitoring to evaluate other types of pollutants and there effects on human health.

CHAPTER 7

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Appendix ١

Questionnaire Sheet

- Name:
- Age:
- weight:

- Smoking habit:
- Place of work:
- Working durations:

Residence:

- Fish meals:

C/O:

History:

1. History of renal colics or pass stones ( )

2. History of hematuria: If present ( )
- Terminal (Prostate)
- All the stream (Kidney stones or tumor)
- At start (schistosomiasis)
- Painless (malignant prostate)

3. History of urine flow abnormalities as ( )
- Urgency (D.M.)
- Frequency (Prostatitis or cystitis)
- Frothy urine (heavy proteinuria)

4. History of jaundice/change color of eye or urine. ( )

5. History of right & left hypochondrial pain or suprapubic pain ( )

6. History of fatty dyspepsia (discomfort after fatty meal) ( )

7. History of fatigue for long period unexplained (HCV) ( )

8. History of schistosomal ( ), HCV ( ), or HBV ( ) infection.

9. History of previous operation ( )

10. History of blood transfusion ( )

11. History of blood in stool: ( )

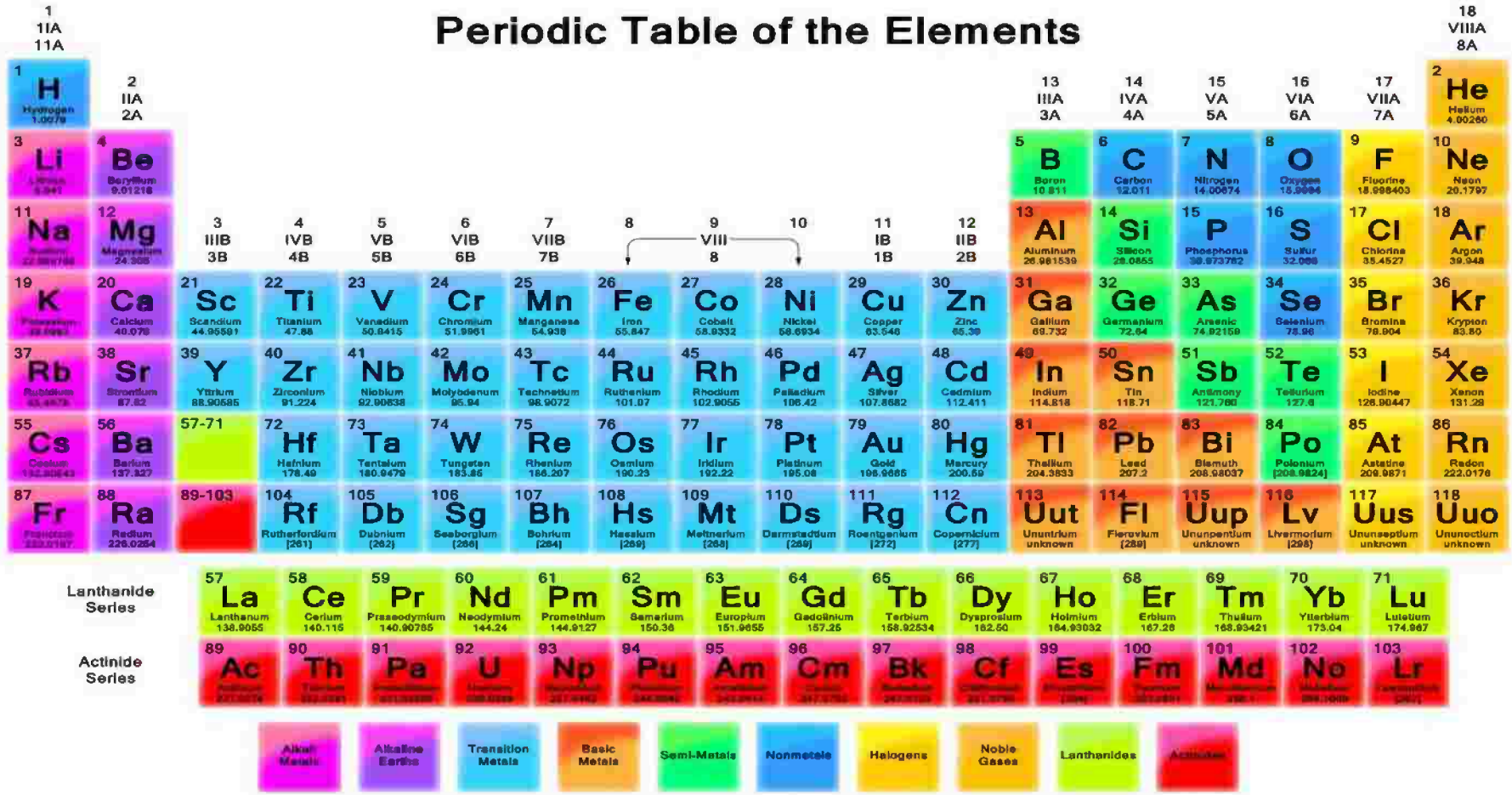
12. History of edema lower limb ( )

13. Echymotic patches over the skin or bleeding tendency as bleeding gums or epistaxsis (hypersplenism) ( )

14. History of D. M. ( )

15. History of hypertension ( )

Periodic Table of the Elements



PROTOCOL

متالوثيونين كدليل حيوي على التلوث بالفلزات و التعبير الجينى لعامل النمو شبيه الأنسولين 2

Metallothionein as a Biomarker of Metals Pollution and Gene Expression of Insulin Like Growth Factor II

Protocol of a thesis submitted
to the Medical Research Institute
University of Alexandria in
partial fulfillment of the
requirements of the degree of

خطة بحث مقدمة إلى
معهد البحوث الطبية
جامعة الإسكندرية
إيفاءً جزئيا لشروط
الحصول على درجة

Ph. D. of Applied Medical Chemistry

الدكتوراه فى الكيمياء الطبية التطبيقية

by

من

Hany Abdel-Hakiem Amin Kassem
M. Sc. Of Applied Medical Chemistry
Medical Research Institute
University of Alexandria
2005

هانى عبد الحكيم أمين قاسم
ماجستير الكيمياء الطبية التطبيقية
معهد البحوث الطبية
جامعة الإسكندرية
2005

Department of Applied Medical Chemistry
Medical Research Institute
University of Alexandria
2007

قسم الكيمياء الطبية التطبيقية
معهد البحوث الطبية
جامعة الإسكندرية
2007

Supervisors

المشرفون

Professor: Aziza Abdel-Azim Ibrahim Saad
Professor, Department of Applied Medical Chemistry
Medical Research Institute
University of Alexandria

الأستاذ / عزيزه عبد العظيم ابراهيم سعد
أستاذ متفرغ بقسم الكيمياء الطبية التطبيقية
معهد البحوث الطبية
جامعة الإسكندرية

Dr: Amany Mohamed Ahmed El-Sikaily
Assist. Prof. of Marine Pollution
National Institute of Oceanography & Fisheries
Ministry of Scientific Research

الدكتور/ أمانى محمد أحمد السقيلى
أستاذ مساعد التلوث البحرى
المعهد القومى لعلوم البحار و المصايد
وزارة البحث العلمى

Dr: Mohamed S. Abdel-Latif
Researcher, Molecular Biology Unit
Medical Technology Center
Medical Research Institute
University of Alexandria

الدكتور/ محمد صلاح الدين عبد اللطيف
باحث بوحدة البيولوجيا الجزيئية
مركز التكنولوجيا الطبية
معهد البحوث الطبية
جامعة الإسكندرية

Background

Water pollution due to toxic heavy metals has been a major cause of concern for chemists and environmental engineers. The development of modern industry has been increasingly responsible for serious pollution in the environment inhabited by humans, with significant health risks, including cancer [1]. In general, the whole soft tissue concentration of metals in marine organisms is many times greater than the concentration of metals in the surrounding seawater. Often, the metal level in organism is proportional to the metal level in seawater so that the organism can be used as a biological indicator of metal pollution [2]. Metals interfere with many cellular reactions. However, the molecular interactions leading to tumor formation after exposure to metals are still not well understood. One mechanism proposed frequently is an increase in oxidative DNA lesions attributable to metal exposure, mediated either by an increased generation of highly reactive oxygen species and/or interference with DNA repair processes [3-5].

During chronic intoxication with heavy metals, liver damage occurs when the capacity of liver to sequester free forms of metals is exceeded [6]. Exposure to hepatotoxins causes liver necrosis, fibrosis, cirrhosis, and subsequently hepatocellular carcinoma [7].

Several different growth factors and their respective receptors have been identified and may act as positive or negative modulators of cell proliferation and differentiation of malignant cell systems. Among these, the insulin-like growth factors I and II (IGF-I and IGF-II), their receptors and their binding proteins play an

increasingly recognized role, being implicated in tumor formation, growth and metastasis in vivo [8]. Most of the circulating IGF-I and IGF-II is produced by the liver, although other tissues are capable of synthesizing these peptides locally [9]. Insulin-like growth factor II (IGF-II) is a mitogenic polypeptide having structural similarity to proinsulin and insulin-like growth factor I (IGF-I) [10]. IGF-II reactivation was reported to be a common phenomenon in hepatocarcinogenesis irrespective of species and the process of hepatocarcinogenesis [10].

Metallothionein (MT) is a low molecular mass metal-binding protein that is inducible by endogenous and exogenous stimuli such as heavy metals and cytokines[11]. The protein consists of (61–68) amino acids, depending upon the isoform, one third of which are cysteine residues without an interamolecular disulfide bond. The cysteine residues are responsible for coordinately binding heavy metals such as cadmium and zinc ions [11]. MTs take part in multiple biological processes. Examples are the homeostasis of essential metals, such as zinc and copper, and the detoxification of toxic metals like cadmium and mercury [12]. Other examples would be the cell protection role against oxidative damage caused by free radicals, pharmacological agents and mutagens, neuroprotection from ionizing radiation, the modulation of the cell apoptosis. The exposure of an organism to toxic factors, such as heavy metals, induces MT expression in different tissues [13, 14]. Therefore, these proteins could be used for exposure assessment in free-living mammals and environmental monitoring of pollution from various metals [12].

Review: To the best of our knowledge several studies were interested in Heavy metals pollution and their role in Carcinogenesis. Here we summarized the most recent publications: Biomonitoring on carcinogenic metals and oxidative DNA damage in a Cross-Section Study (Hiltrud M et al 2001)[3], Molecular mechanisms of metal toxicity and carcinogenesis (Suwei W & Xianglin S 2001)[15], Molecular and cellular mechanisms of cadmium carcinogenesis (Michael W et al 2003)[16], Inhibition of Apel nuclease activity by lead, iron and cadmium (Daniel RM et al 2004) [17], Free radicals, metals and antioxidant in oxidative stress-induced cancer (Valko M et al 2006)[18] and Serum cadmium levels in pancreatic cancer patients from the East Nile Delta region of Egypt (Alison MK et al 2006)[19].

Other studies were interested in Metallothionein as a biomarker of heavy metals pollution, the most recent studies was carried out are: Metallothionein: An intracellular protein to protect against cadmium toxicity (Curtis DK et al 1999)[20], Hepatic metallothionein as a biomarker for metal contamination: age effects and seasonal variation in European flounders (*Pleuronectes flesus*) from the Severn Estuary and Brisol Channel (Rotchell JM et al 2001)[21], A mediator role for metallothionein in Tumor Necrosis Factor-induced lethal shock (Waelput W et al 2001)[22], Metallothionein in liver of eels *Anguilla anguilla* from the Thames Estuary: an indicator of environmental quality (Langston WJ et al 2002)[23], Critical exposure level of cadmium for elevated urinary metallothein-An occupational population study in China (Liang C et al 2006)[24], Metallothionein, antioxidant enzymes and DNA strand breaks as biomarkers of Cd exposure in a marine crab, *Charybdis japonica* (Luqing P & Hongxia Z 2006)[25].

Some studies illustrate the involvement of IGF-II in human cancer some of these literatures are: Altered transcriptional regulation of the insulin-like growth factor 2 gene in human hepatocellular carcinoma (Kosaki U et al 1997)[26] and Role of the insulin-like growth factor family in cancer development and progression (Herbert Y & Thomas R 2000)[27].

This study is considered as a follow-up to fishermen works in El Maadiya region suffering from Chromosomal Aberrations as a result of exposure to aquatic pollution (M. Sc. Thesis by Hany A. Kassem 2005)

The broad long term benefits of the proposed research is surveying the region under study and know if the heavy metals pollution causes alteration in expression of IGF-II gene and this alteration can be used as a suitable marker for very early detection of the cancerous process and can save numbers of future cancer victims by very early detection of this disease.

Where, the literatures did not cited any study on this area and our study considered as the first one interested with the relation between metals pollution and expression of IGF-II gene.

Aim

The ultimate aim of this study is to estimate the Metallothionein concentration as a biomarker of metal pollution and the effect of heavy metals exposure on the gene expression of Insulin-Like Growth Factor II (IGF-II).

Methods

1. *Biological Matrix:* Mussel samples will be collected from Mediterranean Sea "El-Maadiya region" for Determination of Metallothionein [12]. Hence, Mussels have been validating as a good biological matrix for the determination of Metallothionein as a biomarker of response to metal pollution [28].

2. *Human Subjects:* the study will include (50) male subjects with an age range of (21-55) years; they will divided into two groups:

   Group I: which will include (40) professional Fishermen volunteers works in "El-Maadiya region".

   Group II: which will include (10) healthy control volunteers.

   Blood samples will be collected from human subjects for the following investigations:

   - Determination of Metallothionein [29].
   - Determination of Glutathione (GSH) content [30].
   - Determination of Glutathione Peroxidase (GPx) activity [31].
   - Determination of Catalase (CAT) activity [32].
   - Determination of Superoxide Dismutase (SOD) activity [33].
   - Determination of Malondialdehyde (MDA) [34].
   - RNA will be extracted from peripheral blood mononuclear cells by commercially available kit for study of Insulin-Like Growth Factor II (IGF-II) expression level PCR [35].

Analysis of results

The data obtained from this study will be statistically analyzed using SPSS (version 10). One way ANOVA will be used to compare between the levels of the different parameters in study groups. A difference will be considered as significant at $p < 0.05$.

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CHAPTER 8

ARABIC SUMMARY

الملخص العربي

الملخص العربى

أثار تلوث البيئة المائية بالفلزات الثقيلة إهتماماً عالمياً كبيراً. وقد يتم التلوث البيئى بهذه الفلزات عن طريق الترسيب من الغلاف الجوى ، والتآكل الجيولوجي للصخور، بالإضافة للمخلفات الصناعية والزراعية والآدمية ، ولهذه المخلفات آثار ضارة جداً على الأحياء المائية مما يؤثر على صحة الانسان.

وتقاس درجة التلوث بهذه الفلزات بتعين تركيزاتها فى المياه والرواسب البحرية أو النهرية بالإضافة إلى الأحياء المائية. وقد أثبتت الأبحاث العلمية أن تعيين الملوثات فى أنسجة الرخويات تعتبر مؤشر جيداً لقياس درجة التلوث البيئى ، وذلك لشيوع تواجدها فى معظم البيئات الجيوجرافية المختلفة، ولإستقرارها فى أماكنها ، بالاضافة لقوة تحملها لتركيزات عالية من الملوثات . ويعتبر المتالوثيونين من أكثر الدلائل الحيوية المستخدمة للكشف عن تلوث البيئة بالفلزات الثقيلة ، حيث أنه يعتبر دليل حيوى دقيق لإثبات التعرض للفلزات.

ومن هذا المنطلق كان الهدف الأول من هذه الدراسة هو التعيين الكمى لمستوى المتالوثيونين فى أنسجة الرخويات البحرية كدليل حيوى على تلوث خليج أبوقير (منطقة المعدية) بالفلزات الثقيلة.

أظهرت نتائج هذه الدراسة عن تلوث خليج أبوقير (منطقة المعدية) بالفلزات الثقيلة وذلك من خلال وجود نسبة عالية من المتالوثيونين فى أنسجة هذه الحيوانات الرخوية المتواجدة فى هذه المنطقة. وقد أكدت صحة هذه النتائج بوجود بعض من الفلزات الثقيلة فى أنسجة هذه الرخويات مثل الكادميوم والنحاس والكروم والرصاص والزنك.

يعتبر خليج أبوقير حوض مائى شبه دائرى ضحل يقع فى شرق الأسكندرية، حيث يمثل خليج أبوقير أهمية صناعية وتجارية كبرى وسط المناطق الساحلية بجمهورية مصر العربية . يتعرض سكان منطقة المعدية للتلوث البيئى بكميات مباشرة من الفلزات الثقيلة، مما جعل الهدف الثانى لهذه الدراسة هو دراسة معدل المخاطر المعرض لها سكان هذه المنطقة بسبب تلوث خليج أبوقير، وأثرها على صحة الانسان، من خلال تعيين بعض أنواع من الفلزات الثقيلة، وذلك بتعيين تركيز المتالوثيونين فى دم الصيادين محل البحث، ودراسة أثر التعرض لهذه الفلزات على ضغوط الأكسده فى جسم هؤلاء الصيادين من خلال تعيين تركيزات بعض مضادات الأكسدة منها محتوى الجلوتاثيون ، وإنزيم الكاتالاز، وإنزيم الجلوتاثيون بيرأوكسديز وإنزيم السوبرأكسيد ديسميوتيز بالإضافة إلى تعيين المالون دى ألدهيد فى دم هؤلاء الصيادين وتأثير هذا على مستوى التعبير الجينى لعامل النمو شبيه الأنسولين ٢.

تمت هذه الدراسه على 56 شخص مقسمة إلى مجموعتين:

المجموعة الأولى (المجموعة الضابطة) وتشمل 12 أشخاص يعملون فى مهن مختلفه غير الصيد

والمجموعة الثانية تتضمن 44 صياداً محترفاً فى منطقة المعدية .

أسفرت نتائج هذه الدراسة عن وجود تركيزات عالية من الفلزات الثقيلة مثل (الكادميوم – الكروم – رصاص – نحاس – زنك) فى دم الصيادين محل البحث مصاحب لإرتفاع معنوى ملحوظ لمستوى المتالوثيونين فى خلايا الدم الحمراء الخاصة بالصيادين عنها فى المجموعة الضابطة.

يدخل المتالوثيونين فى عمليات التوازن الحيوى للفلزات الضروريه فى جسم الانسان منها (النحاس - الزنك) ، حيث يعتبر بروتين أساسى مرتبط بالنحاس والزنك فى معظم أنسجة الجسم، و الزياده الكبيره فى المتالوثيونين يؤدى إلى تثبيط الموت المبرمج للخلايا.

وقد أثبتت نتائج هذه الدراسة أن تعرض الصيادين لأنواع مختلفة من الفلزات الثقيلة تنج عنه حالة من ضغوط الأكسد الخطيرة، والتى أثبتت من خلال وجود مستوى معنوى عالى من المالون دى ألدهيد مصاحب بإنخفاض معنوى كبير فى معدلات مضادات الأكسدة فى دم هؤلاء الصيادين (انخفاض معنوى كبير فى مستوى الجلوتاثيون والجلوتاثيون بيرأوكسديز والكاتالاز).

ويعتبر زيادة ضغوط الأكسدة بالجسم الناتج من التعرض للفلزات سببا لحدوث الطفرات الجينية المسببة للسرطان. وأظهرت نتائج دراسات سابقة أن الفلزات تساعد على تكوين جزئيات الأوكسجين النشطة فى الخلايا وتكوين جزئ الهيدروكسيل النشط ، حيث وجد أن هه الجزيئات النشطة تساهم بدورها فى تكسير الدهون والبروتينات وكذلك الأحماض النووية فى الخلية. وتتسبب الفلزات فى تغير التعبير الجينى عن طريق تداخلاتها مع الإشارات الخلوية المتبادلة التى بدورها مسئولة عن نمو الخلية وتطورها.

وجدير بالذكر أن نتائج أبحاث بقسم الكيمياء الطبية التطبيقية أثبتت تلوث منطقة المعدية بالهيدروكربونات الحلقية العطرية (رسالة ماجستير) ومواد عطرية أمينية (رسالة ماجستير)، كما أثبتت نتائج هذه الدراسة القائمة عن تلوث ذه المنطقة بالفلزات الثقيلة. وقد أسفرت نتائج هذه الأبحاث عن حدوث ضغط تأكسدى معنوى خطير عند صيادين هذه المنطقة، بالاضافة لحدوث زيغ كروموسومى معنوى كبير عند هؤلاء الصيادين، كما أكدت نتائج الدراسات الحالية عن حدوث إرتفاع معنوى ملحوظا فى مستوى التعبير الجينى لعامل النمو شبية الانسولين ٢ فى دم صيادين نفس المنطقة. ولعامل النمو شبية الانسولين ٢ دور كبير فى عمليات تنظيم نمو الخلايا الكبدية والتفاعلات الأيضية بها، كما يعتبر التغيير فى التعبير الجينى لعامل النمو شبية الانسولين ٢ مقياس لكفاءة هذه الخلايا. وقد أثبتت نتائج أبحاث سابقة أن تغيير التعبير الجينى لعامل النمو شبية الانسولين ٢ دليلاً حيوياً جيداً للتشخيص المبكر للأورام السرطانية الكبدية، عليه ممكن القول بإحتمال وجود إصابات كبدية كامنة بدون أعراض عند هؤلاء الصيادين محل الدراسة.

وقد أثبتت بعض الأبحاث السابقة على أن أيونات الفلزات تحدث خللاً فى الموت المبرمج للخلايا وتعمل عن طريق تثبيط بروتين p٥٣ مما يطيل من عمر الخلية ويجعلها قابلة للتحول السرطانى . وتزداد الأدلة على وجود تداخلات بين عامل النمو شبية الانسولين ٢ و p٥٣ فى عملية تكوين الخلية السرطانية. وحديثا وجد أن زيادة التعبير الجينى لعامل النمو شبية الانسولين ٢ يؤدى إلى تكوين الأورام بواسطة تثبيط نشاط p٥٣ .

بناءاً عليه، يعتبر وجود النواتج البولية للأمينات العطرية والهيدروكربونات العطرية الحلقية بالتزامن مع وجود فلزات الثقيلة فى دم الصيادين بالأضافة لتعرضهم لجهد تأكسدى عالى ومصاحب بزيادة فى مستوى المتالوثيونين والزيغ الكروموسومى وزيادة التعبير الجينى لعامل النمو شبية الانسولين ٢ يجعل صيادين وساكنى منطقة المعدية عرضة مؤكدة لمخاطر حدوث أورام سرطانية فى المستقبل .

التوصيات

١. ضرورة عدم إلقاء المخلفات الآدمية و الصناعية فى البحار او البحيرات و ضرورة المعالجة الجيده للمخلفات قبل صرفها.

٢. أهمية عمل متابعة دورية لهؤلاء الصيادين كل ستة أشهر بعمل تحاليل و أشعة للكشف المبكر على ظهور اى أمراض أو أورام.

٣. أهمية التركيز على إجراء أبحاث مستقبلية على عامل النمو شبية الانسولين ٢ لمعرفة آلية يداخلة مع باقى المحتوى الخلوى على مستوى الأنسجة لمعرفة دورة فى عملية تكوين الخلية السرطانية.

٤. لابد من إجراء أبحاث مستقبلية لمعرفة مدى الأهمية التطبيقية لتعيين التعبير الجينى لعامل النمو شبية الانسولين ٢ كدليل مبكر لحدوث أورام سرطانية.

٥. ضرورة عمل دراسات مسْحيّة لمنطقة المعدية للوقوف على مستوى تلوث هذه المنطقة بالملوثات الأخرى.

جامعة الإسكندرية
معهد البحوث الطبية
قسم الكيمياء الطبية التطبيقية

متالوثيونين كدليل حيوي على التلوث بالفلزات و التعبير الجينى لعامل النمو شبيه الإنسولين ٢

رسالة مقدمة
بقسم الكيمياء الطبية التطبيقية – معهد البحوث الطبية – جامعة الإسكندرية
ضمن متطلبات درجة

دكتوراه الفلسفة
فى
الكيمياء الطبية التطبيقية
من

هانى عبد الحكيم امين قاسم
ماجستير الكيمياء الطبية التطبيقية،٢٠٠٥
معهد البحوث الطبية
جامعة الإسكندرية

٢٠١٥

لجنة الإشراف **موافقون**

أ.د/ عزيزه عبد العظيم ابراهيم سعد .....
أستاذ متفرغ الكيمياء الحيوية
قسم الكيمياءالطبية التطبيقية
معهد البحوث الطبية
جامعة الإسكندرية

أ.د/ أمانى محمد أحمد السقيلى .....
أستاذ و رئيس معمل التلوث البحرى
المعهد القومى لعلوم البحار و المصايد
وزارة البحث العلمى

د/ محمد صلاح الدين عبد اللطيف .....
مدرس الميكروبيولوجى
كلية العلوم الطبية المساعدة
جامعة فاروس بالاسكندرية

جامعة الإسكندرية
معهد البحوث الطبية
قسم الكيمياء الطبية التطبيقية

متالوثيونين كدليل حيوي على التلوث بالفلزات و التعبير الجينى لعامل النمو شبيه الإنسولين ٢

رسالة مقدمة من

هانى عبد الحكيم امين قاسم

للحصول على درجة دكتوراه الفلسفة

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التاريخ: ٢٤ / ١ /٢٠١٥