Serum Concentration of Thyroid Hormones Long-Term After Sulfur Mustard Exposure

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Abstract

Background: Despite several reports on the clinical manifestations of sulfur mustard (SM) intoxication, there is no study on serum concentrations of thyroid hormones long-term after SM exposure. In this study, the changes in thyroid functioning parameters 20 yr after SM exposure were evaluated.

Methods: This study is a part of a larger historical cohort study conducted in 2007 following 20 years of the exposure to SM, called Sardasht–Iran cohort study (SICS). We (SICS) comprised an SM–exposed group from Sardasht City, West Azerbaijan Province, Iran (n=169 as hospitalized group and n=203 as non-hospitalized exposed group); and control participants were selected from Rabat, a town near Sardasht (n=126). Peripheral blood samples were taken in fasting state and then the sera were separated. T4, T3, TSH, antithyroglobulin (anti–Tg), and antithyroid peroxidase (anti–TPO) concentrations in the sera were measured by the ELISA method.

Results: The mean of T3 concentration was significantly higher in the exposed than control group (0.88 ± 0.26 nmol/L vs 0.8 ± 0.25 nmol/L, P<0.001). The levels of TSH, T4, and T3up were not significantly different between the exposed and control groups. Thyroglobulin level was significantly higher in the exposed non-hospitalized group (56.07 ± 140.22 µg/L vs 17.66 ± 41.49 µg/L, P=0.004), but the level of anti–Tg and anti–TPO showed no significant differences between the two groups.

Conclusion: More studies are needed on the alterations in thyroid hormones, their gene expressions, and mechanisms involved in SM exposure to clarify the causes of these alterations.

Keywords: Sulfur mustard; Serum; Thyroid hormones

Introduction

Sulfur mustard [bis(2–chloroethyl) sulfide; SM], also known as mustard gas, was initially used as a vesicant chemical warfare agent during World War I. According to the report of the specialists appointed by the Secretary-General of the United Nations, mustard gas was extensively used by Iraq during the conflict against Iran (1). Sardasht, a border town in the northwest of Iran, was exposed to sulfur mustard in 1987 by the Iraqi army. In addition to acute extensive multi-
organ problems, there are long-term adverse health effects of exposure to mustard gas (2-4).

Despite a fair number of reports on clinical manifestations of SM intoxication, there are few studies on paraclinical and molecular parameters and systemic effects of SM long term after exposure. Therefore, the underlying molecular mechanisms are less known.

Various complaints and clinical presentations of the survived people seeking medical care and review of the literature support such concerns, even though no systematic scientific evidence has been available (5).

A decline in mean serum–free T4 index (FT4I) and free T3 index was reported less than one month after exposure (6). Moreover, they explained the time course of serum concentration of thyroid hormones. Free T4 and T3 indices decreased and reverse T3 (rT3) increased in the first week following exposure. Except for an increase in FT4I and a decrease in thyroid–stimulating hormone (TSH) by the third week, serum hormone concentrations remained unchanged until the fifth week after injury. They concluded that exposure to chemical weapons containing SM results in alterations in serum concentrations of thyroid hormones.

There is no study on serum concentrations of thyroid hormones long-term after SM exposure. In this study, the changes of thyroid function parameters 20 years after SM exposure were evaluated.

Materials and Methods

Study Design and Participants

This study is a part of a larger historical cohort study conducted in 2007 following 20 years of the exposure to SM, called Sardasht, West Azerbaijan Province, Iran cohort study (SICS), of which the details of the study design and methods have been previously reported (7).

Briefly, the exposed group were male individuals from Sardasht categorized into two major subgroups based on severity of injuries, just after exposure. SM–exposed patients hospitalized based on the severity of injuries after acute exposure were considered as the hospitalized group (n=169) who had a history of at least one–week admission in hospital, and patients with mild and subclinical problems not hospitalized after acute exposure as the non-hospitalized exposed group (n=203).

Control participants were selected from Rabat, a town near Sardasht, concurrently with the exposed group. Moreover, the control samples included men matched with the study group by age (n=126). The age range covered by the study was 20–60 yr. Both study groups were compared in terms of marital status, level of education, employment, and smoking status.

Ethical approval

The study was approved by the Ethical Committee of the Board of Research Ethics of Janbazan Medical and Engineering Research Center (JMERC), the Board of Research of the Ministry of Health and Medical Education, and Shahed University. Volunteers who signed an informed consent were recruited.

Serum Preparation

Peripheral blood samples were taken in the morning in fasting state using Vacationer tubes (BD Biosciences), 20 years following SM exposure in the exposed group and, alongside, in the control group. After clotting, the sera were separated by a 20 min centrifugation at 2000×g in 4 °C, aliquoted and stored at −80 °C until test.

Measurement of Thyroid Hormones

T3, T4, and TSH were measured by enzyme-linked immunosorbent assay (ELISA) method (DBC, Canada). Intra- and inter-assay coefficients of variations (CV) were 3.3% and 6.2% for T4, 12.3% and 10.4% for T3, and 3.9% and 7.1% for TSH, respectively.

According to the manufacturer’s instructions, the normal range was 0.52–1.85 ng/ml for T3, 4.4–10.8 µg% for T4 and 0.4–6.1µIU/ml for TSH.

Antithyroglobulin (anti–Tg) and anti-thyroid peroxidase (anti–TPO) concentrations were measured by ELISA (Radim, Pomezia, Italy).

The values of anti–TPO, up to 150IU/ml and anti–Tg, up to 75IU/ml were considered normal. The inter- and intra-assay CVs were 7.2% and 6.5%, respectively.
**Statistical Analysis**

Statistical analysis was done using the SPSS (Windows, ver. 16.0, Chicago, IL, USA). Values are given as mean (± SD). Comparison of thyroid factors between study groups was done with ANOVA (Tukey’s post hoc). Comparison of the normal range was done with chi-square test. A $P$-value of less than 0.05 was considered statistically significant.

**Results**

The mean age of the exposed group was 44 ± 11 yr. The result revealed insignificant differences in terms of age, marital status, and smoking status in contrast to significant differences in terms of level of education and, and employment, which were higher among the exposed group (Table 1).

### Table 1: Demographic Characteristics of Study Groups

|                | Study Groups | Control | %  | Exposed | %  | $P$-value |
|----------------|--------------|---------|----|---------|----|-----------|
| Age (yr)       |              |         |----|---------|----|-----------|
| <40            |              | 57      | 46.0| 126     | 35.3| 0.097     |
| 40 - 50        |              | 35      | 28.2| 113     | 31.7| 0.866     |
| >50            |              | 32      | 25.8| 118     | 33.1|           |
| Marital status |              |         |----|---------|----|-----------|
| Not-married    |              | 9       | 7.6 | 28      | 8.0 |           |
| Married        |              | 110     | 92.4| 320     | 92.0|           |
| Education      |              | 93      | 78.2| 201     | 57.9| <0.001    |
| Higher diploma |              | 26      | 21.8| 146     | 42.1|           |
| Diploma and up |              | 241     | 86.6| 325     | 87.2|           |
| Occupation     |              |         |----|---------|----|-----------|
| Employed       |              | 52      | 44.1| 210     | 60.5| 0.002     |
| Unemployed     |              | 66      | 55.9| 137     | 39.5|           |
| Smoke          |              | 29      | 22.8| 84      | 22.6| 0.573     |
| Yes            |              | 98      | 77.2| 287     | 77.4|           |

### Table 2: Thyroid hormones levels in Sulfur mustard Exposed and control groups

| Study Groups | T3 ng/ml | T4 mg/dl | TSH mIU/L | T3% uptake | Tg ng/mL | anti Tg IU/ml | anti TPO IU/ml | Free T4 Index | Free T3 Index |
|--------------|----------|----------|-----------|------------|----------|--------------|---------------|---------------|---------------|
|              | Unexposed| Exposed  | Unexposed | Exposed    | Unexposed| Exposed      | Unexposed     | Exposed       | Unexposed     |
| N            | 127      | 202      | 371       | 127        | 202      | 169          | 371           | 127           | 202           |
| Mean         | 229.50   | 229.16   | 77.2      | 1.41       | 8.02     | 82.34        | 82.34         | 229.00        | 229.00        |
| Median       | 226.20   | 226.56   | 77.4      | 1.10       | 8.10     | 82.34        | 82.34         | 226.00        | 226.00        |
| P-value<sup>1</sup> | 0.005    | 0.018    | 0.665     | 0.605      | 0.080    | 0.046        | 0.046         | 0.712         | 0.712         |
| P-value<sup>2</sup> | 0.924    | 0.395    | 0.110     | 0.036      | 0.825    | 0.071        | 0.071         | 0.460         | 0.460         |

<sup>1</sup> Comparison of Control (ANOVA and t-test)
<sup>2</sup> Comparison between hospitalize and non-hospitalized (ANOVA Tukey Post hoc)
The details of hormonal assessment are presented in Tables 2 and 3. Serum T4 (μg/dL) was 7.95 ± 1.44 (range: 4.4-10.8 μg%) and serum TSH was 1.41 ± 2.06 (range: 0.4-6.1 μIU/ml) in the exposed group. T4 was 8.24 ± 1.78 (range: 4.4–10.8 µg%) and TSH was 1.47 ± 2.19 (range: 0.4–6.1 μIU/ml) in the control group.

The mean of T3 concentration was significantly higher in the exposed group (0.88±0.26 nmol/L) compared to the control (0.80 ± 0.25) (P<0.001); however, these values were still within normal clinical range. The levels of TSH, T4, T3 up, anti–Tg and anti–TPO were not significantly different between the exposed and control groups. Regarding two subgroups of exposure (hospitalized and non-hospitalized), the patterns of measured parameters were similar. Thyroglobulin level was higher in the exposed but not hospitalized group (56.07±140.22 μg/L, P=0.004), compared to the control group. The free T3 index, calculated from the serum total of T3 and T3 uptake, was significantly elevated in the exposed group similar to the T3 level.

The results of this study showed that the ratio of T3 to T4 in the exposed group was significantly higher than the control group (P= 0.006) (Table 4).

| Variable | Unexposed N | % | Non-Hospitalized N | % | P-value | Hospitalized N | % | P-value | Exposed N | % | P-value |
|----------|-------------|---|-------------------|---|---------|---------------|---|---------|-----------|---|---------|
| T3 level (0.52 - 1.85) | < 0.52 | 15 | 11.8 | 10 | 5.0 | 6 | 3.6 | 16 | 4.3 |
| | Normal | 111 | 87.4 | 189 | 93.6 | 0.065 | 162 | 95.9 | 0.023 | 351 | 94.6 | 0.010 |
| | > 1.85 | 1 | .8 | 3 | 1.5 | 1 | .6 | 4 | 1.1 |
| T4 Level (4.4 - 10.8) | < 4.4 | 1 | .8 | 4 | 2.0 | 1 | .6 | 5 | 1.3 |
| | Normal | 121 | 95.3 | 193 | 95.5 | 0.528 | 165 | 97.6 | 0.513 | 358 | 96.5 | 0.494 |
| | > 10.8 | 5 | 3.9 | 5 | 2.5 | 3 | 1.8 | 8 | 2.2 |
| TSH Level (0.4 - 6.1) | < 0.4 | 21 | 16.5 | 22 | 10.9 | 30 | 17.8 | 52 | 14.0 |
| | Normal | 104 | 81.9 | 175 | 86.6 | 0.300 | 139 | 82.2 | 0.256 | 314 | 84.6% | 0.767 |
| | > 6.1 | 2 | 1.6 | 5 | 2.5 | 0 | .0 | 5 | 1.3 |
| T3 up Level (25 - 38) | < 25 | 7 | 5.5 | 6 | 3.0 | 7 | 4.1 | 13 | 3.5 |
| | Normal | 120 | 94.5 | 196 | 97.0 | 0.249 | 162 | 95.9 | 0.583 | 358 | 96.5 | 0.320 |
| | > 38 | 0 | .0% | 0 | .0 | 0 | .0 | 0 | .0 |
| anti Tg 50-75 | lower 75 | 82 | 88.2 | 112 | 83.6 | 0.335 | 91 | 92.9 | 0.268 | 203 | 87.5 | 0.868 |
| | 75 and up | 11 | 11.8 | 22 | 16.4 | 7 | 7.1 | 29 | 12.5 |
| anti TPO 100-150 | lower 150 | 89 | 94.7 | 126 | 95.5 | 0.790 | 95 | 96.0 | 0.674 | 221 | 95.7 | 0.700 |
| | 150 and up | 5 | 5.3 | 6 | 4.5 | 4 | 4.0 | 10 | 4.3 |

Table 3: Comparison of thyroid hormone levels between study groups according to the normal levels

| Variable | Study Groups | N | Mean T3/T4 | Standard Deviation | Median T3/T4 | P-value |
|----------|--------------|---|------------|-------------------|--------------|---------|
| T3 level | Control | 127 | .0100 | .00356 | .0100 | 0.006 |
|          | Exposed | 371 | .0115 | .00577 | .0108 |       |

Table 4: The ratio of T3 to T4 in study group

Discussion

The aim of this study was to determine the circulating levels of thyroid hormones 20 years after SM exposure. The mean concentrations of T4 and TSH in the exposed group were not significantly different from the control group. Whereas serum T3 concentrations and T3/T4 ratio exhibit...
The delayed toxic effects of SM were documented in Iranian veterans, focusing on head and neck complications. For the first time, they reported carcinomas of thyroid and nasopharynx in patients with SM exposure (8). Despite the decrease in T3 level in most of these reports, different elucidations could interpret the elevation of T3 in our study; one is that more subjects in our control group have lower level of T3 than normal range (11.8% in control and 4.3% in exposed). On the other hand, there are numerous human and animal studies on the variety of chemicals that disrupt thyroid hormone homeostasis. Such differences in T3 and T3/T4 ratio were observed in exposure to contaminant chemicals such as PCBs in fish (9).

In vertebrates, the main hormone produced by thyroid, prohormone T4, is converted to T3 in peripheral target tissue cells by 5-deiodinase (10). The differences in T3 and T3/T4 ratio may point to alterations in the peripheral conversion of T4 into T3. Some studies on fish suggested that pesticides and other chemicals may alter 5-deiodinase activity, which can lead to increased T3 and decreased T4 (4-6, 11-13). Although there has been no study on the cellular and molecular mechanisms involved in SM-induced thyroid function disorders, there are a large number of studies on endocrine-disrupting chemicals (EDCs).

Numerous mechanisms have been proposed, including changes in the expression of a large number of potential target genes or proteins, altered by EDCs (14). Activation of EDCs may interfere with thyroid function by interfering with thyroid hormone synthetic pathways, deiodinase functions in peripheral tissues, altering TH turnover, and carrying proteins in the blood (6, 8, 13, 15-19).

However, the limitation of this study is that as our subjects were selected from a previous large cohort study, detailed clinical evaluation in view of specific thyroid assessment was not performed. Further insight into the role of SM in the pathogenesis of thyroid requires deep researches on the cellular and molecular mechanisms involved. This would enable both researchers and
clinicians to provide more accurate evaluation and therapeutic strategies when dealing with sufferers of SM exposure. More studies are needed on the alterations in thyroid hormones, their gene expressions, and involved mechanisms in SM exposure to clarify the causes of these alterations.

**Ethical considerations**

Ethical issues (including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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**Conflict of interest**

The authors report no conflict of interest in this study.

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