An Epidemiologic Study to Screen for Chronic Myelocytic Leukemia in War Victims Exposed to Mustard Gas

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Chemical agents such as mustard gas (or sulfur mustard), which has alkylating characteristics, were used against Iranian combatants in the Iraq–Iran war. Previous studies have not shown a strong link between these chemical agents and the development of chronic myelocytic leukemia (CML). The purpose of this study was to evaluate the increased risk of CML development in Iranian soldiers exposed to mustard gas during the war. Based on a descriptive study of 2,500 cases with documented exposure to various chemical warfare agents, 665 cases had documented exposure to mustard gas. We screened the latter using the leukocyte alkaline phosphatase (LAP) test and performed further cytochemical studies on cases with positive results. From among the 665 cases with documented exposure to mustard gas, 9 cases had LAP scores < 20; 2 of these 9 cases had CML and a score of zero (0.3%). We detected cytogenetic abnormalities in 7 patients with low LAP scores and atypical lymphocytes of 5–11% in 40 patients. The risk ratio of CML developing in victims exposed to mustard gas (cutaneous or respiratory) may be higher in comparison with the normal population, although confounding factors (e.g., the possibility of exposure to combined chemical agents, excluding patients who did not manifest blisters) limited our results. Because the increased development of CML in young patients with a documented history of exposure to mustard gas cannot be disregarded, further studies are needed. Key words: chronic myelocytic leukemia, Iran, mustard gas, war victims.

Materials and Methods

Thousands of Iranian soldiers were exposed to mustard gas during the Iraq–Iran war from 1983 to 1989 (4). Mustard gas, also known as sulfur mustard (SM), is an alkylating agent with mutagenic effects (2). Mustard gas became recognized as the first of a new class of toxins known as “blistering agents.” Skin contact, which could occur via airborne concentrations that penetrate easily through most clothing, led to damage ranging from a sunburnlike scald to bullous necrosis. Eye contact with SM caused severe corneal injury, and inhalation induced acute tracheobronchitis with massive epithelial sloughing (3).

Previous studies have shown that the risk of lung cancer increases after long-term, low-level exposure to mustard gas (4). There have been no reports of chronic myelocytic leukemia (CML) following SM exposure or of a confirmed relationship between alkylating agents and CML (5). According to data from one of the military health centers, two cases of CML have been reported in 2 consecutive years in soldiers who had a history of mustard gas exposure during the war. We therefore undertook this study to seek a relationship between SM exposure and the development of CML.

Results

The mean ages (± SD) were 34 ± 3 years for the patients and 34 ± 1.2 for the control group. The average time lapse from injury was 12 ± 2 years. Table 1 indicates the means of the blood indices of the two groups, along with the statistical results. The mean LAP score of the patients was 59.9 ± 18.2. Nine cases had LAP scores < 20, and two cases had a score of 0 and were reported as CML cases; the other data for these two cases are shown as cases 1 and 2 in Tables 2 and 3. The CBCs with differentiation, Philadelphia chromosome, and serum zinc level for these patients are listed in Table 2. In case 1, the Philadelphia chromosome was positive with typical laboratory manifestations of CML, the LAP score was 0, peripheral blood smears had high white blood cell counts and a shift to the left compatible with CML. Case 2 also showed cellular morphology of peripheral blood smears and bone marrow typical of CML and low serum zinc but was negative for the Philadelphia chromosome. The cytogenetic findings of these two cases and others with low LAP scores are shown in Table 3. From among the nine cases affected by SM, we found seven cases (77%) of karyotypic clones that were diploid and two cases (23%) that were pseudodiploid, including one with a score of 0 and were reported as CML cases; the other data for these two cases are shown as cases 1 and 2 in Tables 2 and 3. The CBCs with differentiation, Philadelphia chromosome, and serum zinc level for these patients are listed in Table 2. In case 1, the Philadelphia chromosome was positive with typical laboratory manifestations of CML, the LAP score was 0, peripheral blood smears had high white blood cell counts and a shift to the left compatible with CML. Case 2 also showed cellular morphology of peripheral blood smears and bone marrow typical of CML and low serum zinc but was negative for the Philadelphia chromosome. The cytogenetic findings of these two cases and others with low LAP scores are shown in Table 3. From among the nine cases affected by SM, we found seven cases (77%) of karyotypic clones that were diploid and two cases (23%) that were pseudodiploid, including one with
the classic Philadelphia chromosome. In all of nine cases, the other minor clones (Table 3) accompanied the major clonal abnormalities. Three of these had hyperdiploid clones.

### Discussion and Conclusion

Acute complications of SM on the hematopoietic system have been reported repeatedly during the Iran–Iraq war (7–9). Aplastic anemia resulting in death, as well as reversible patterns, has also been documented (8,10). We have found no published data documenting CML development after exposure to SM. In the cases in our study, SM exposure was documented because of blisters on the skin and long-term respiratory disorders, symptoms caused by no other chemical warfare agent. In our study group, blood indices revealed significant differences with the control group because of the large sample, but this was not meaningful clinically. Although victims with severe respiratory exposure to SM do not survive, victims do survive when exposure is not excessive. Patients with fever, active infectious disease, or a history of drug use (i.e., ACTH, adrenal 17-OH-corticosteroids) were excluded from this study because these factors have been shown to affect the LAP test (11).

CML is a disease of the elderly, and its peak incidence is usually in the fifth and sixth decades of life. The annual incidence of CML is about 1 per 100,000, and it appears to be constant worldwide. From among 2,500 cases with documented exposure to various chemical warfare agents, 665 patients had documented exposure to mustard gas. We found two cases with CML in 2 consecutive years. Although we could not ascertain whether combined chemical gases were used along with SM in the study group, we verified exposure to this gas in all cases by the aforementioned inclusion criteria. In our study, we chose the control group from among the normal population and not from war veterans because low-dose exposure without significant symptoms may have been present among these soldiers. If we accept only the case with positive Philadelphia chromosome as a CML case, then this may be a sporadic case with no relation to mustard gas exposure. However, if we consider it as an outcome of SM, then the occurrence rate is 1 case per 2,500, and this prevalence is 400 times greater than that in the normal population. These findings compelled us to evaluate all of SM-exposed victims for leukemia in a well-designed study that should be completed by 2004.

LAP scoring is a method that is widely used in diagnostic hematology. This method establishes a discriminating factor between CML and other conditions with leukemoid reactions (12,13). LAP is consistently reduced in persons with CML, paroxysmal nocturnal hemoglobinuria (PNH), and other paroxysmal reactions. The constant worldwide. From among 2,500 cases with documented exposure to various chemical warfare agents, 665 patients had documented exposure to mustard gas. We found two cases with CML in 2 consecutive years. Although we could not ascertain whether combined chemical gases were used along with SM in the study group, we verified exposure to this gas in all cases by the aforementioned inclusion criteria. In our study, we chose the control group from among the normal population and not from war veterans because low-dose exposure without significant symptoms may have been present among these soldiers. If we accept only the case with positive Philadelphia chromosome as a CML case, then this may be a sporadic case with no relation to mustard gas exposure. However, if we consider it as an outcome of SM, then the occurrence rate is 1 case per 2,500, and this prevalence is 400 times greater than that in the normal population. These findings compelled us to evaluate all of SM-exposed victims for leukemia in a well-designed study that should be completed by 2004.

### Table 3. Cytogenetic findings and distribution of karyotypic cell lines in nine male patients.

| Case | Hyperdiploidy | Pseudodiploidy | Mitoses with chromatid breaks | Diploidy | Diagnosis | Age (years) |
|------|---------------|----------------|------------------------------|----------|-----------|-------------|
| 1    | —             | 46/XY/—        | 46/XY [5]                    | CML      | 42        |
| 2    | 46/XY/including 2 fragments [2] | 46/XY,22q(-)[4] | 3q/6q/14q [6] | 46/XY [12] | CML | 31 |
| 3    | —             | 46/XY 22q(-)   | —                            | 46/XY [12] | Healthy | 32 |
| 4    | 47/XY/22q [4] | 46/XY/—/1+ marker/22q [—] | — | 46/XY [10] | Healthy | 45 |
| 5    | —             | 46/XY/including fragments [13] | — | 46/XY [4] | Healthy | 42 |
| 6    | 46/XY/—/19+marker [5] | — | — | 46/XY [10] | Healthy | 34 |
| 7    | 47/XY/1+2 [3] | 47/XY/—/1+4 [1] | — | 46/XY [12] | Healthy | 37 |
| 8    | —             | 46/XY/including 2 fragments [2] | — | 46/XY [12] | Healthy | 36 |
| 9    | 47,Xy,+13 [2] | 46,Xy,+16,+18,21 [1] | 3q,6q,14q and 9 gaps (ch 14) [6] | 46/XY [20] | Healthy | 29 |

### Abbreviations:
- ch, chromosome; pter, end of short arm of chromosome. The numbers inside the brackets indicate the number of cells.
hemoglobinuria, and hypophosphatasia, and can be used to diagnose these disorders. Low levels of LAP have also been reported in thrombotic thrombocytopenic purpura, aplastic anemia, and sickle cell disease. We ruled out these conditions in seven cases without CML by clinical and laboratory review of the findings. Although it is not obvious why LAP scores decreased, this decrease may be related to the presence of other cytogenetic abnormalities that are present in these patients, and follow-up studies should determine that. Previous studies have shown that the LAP test is a first-line test for CML and generally precedes a diagnostic algorithm that also includes bone marrow biopsy, cytogenetic analysis, and molecular diagnostics (14). Although bcr and abl had helped to definitively confirm CML in our cases, we did not find a laboratory expert in this analysis at the time of our study. The Philadelphia translocation results in the fusion of the bcr and abl genes in a head-to-tail fashion. The bcr gene loses a 3' section, which is replaced by a 3' abl sequence from chromosome 9.

This study also shows that the LAP test can be used as a screening test for detection of CML in high-risk patients. Previous reports have shown that ionizing irradiation (15) and occupational exposure to nonionizing radiation through electrical work are linked particularly to CML (16). Benzene exposure is also acknowledged to increase the risk of myeloid leukemia (17) and case reports have linked CML with ingestion of cytotoxic drugs (18). Because of failure to follow up thousands of World War II veterans (in Australia, the United States, and Canada) exposed to SM, there is no report regarding CML development. To our knowledge, this is the first case report that links inhalation of SM, an alkylating agent, with the development of CML. Our findings indicate that the incidence of CML among war veterans is significantly greater than in the general population.

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