IJMCM
Winter 2012, Vol 1, No 1

Original Article

# Cleft Palate induced by Sulfur Mustard in mice fetus

**Mohammad Hassanzadeh-Nazarabadi [1*], Nasrin Sanjarmoosavi [1], Naser Sanjarmoosavi [1], Sahar Shekouhi [1]**

[1]*Medical Genetics Department, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

Sulfur Mustard (SM) is a chemical warfare agent which was widely used in the World War I and more recently during Gulf war in the early 1980s'. SM is a strong alkylating agent with known mutagenic and carcinogenic effects; but only few studies have been published on its teratogenicity. Since SM has been widely used as a chemical weapon by the Iraqi regime against the Iranian soldiers as well as the civilian population particularly pregnant women in the border area; therefore, the investigation of SM adverse effects on cleft malformations which is one of the most frequent congenital anomalies is considered in this study. An experimental work has been carried out in embryopathy in mouse with intraperitoneal injection of 0.75 and 1.5 mg/kg SM at different periods of gestation. Cleft lip and palate were examined by stereomicroscopy. Current data demonstrate that exposure with SM on the 11th day of gestation can increase the incidence of cleft defects in comparison with control group ($P<0.001$). These results also show that SM treatment in GD 11 and 13 can lead to more anomalies compared with GD 14 ($P<0.001$). They also show that the teratogenic effects of SM are restrictively under the influence of the threshold dose and time of gestation. The present results suggest that exposure to sufficient doses of SM on critical days of gestation may increase the risk of congenital cleft malformations.

**Keywords:** Sulfur Mustard, teratogenicity, cleft lip/palate

In World War I (1914-1918), the use of chemical weapons especially mustard gas (SM) led to thousands of death (1,2,3). Without attention to the conventional laws that prohibit the use of these weapons, these agents were applied by the Iraqi Army during the Gulf war (1981-1989) which caused the deaths of many soldiers (1).

The destructive effects of SM are well recognized. The eyes, the skin, and the respiratory tract are the principal organ targets of SM toxicity (4-8). SM is highly lipophilic and is absorbed very quickly through the skin. After a latent period of 6-24 h erythema and blisters appear on the skin (6). Pulmonary complications mainly on the upper respiratory tract such as hemorrhagic inflammation, sore throat, hoarseness, cough, bronchitis, and bronchopneumonia are observed in SM-exposed victims (6,9). Additionally, lung cancers had been reported in fishermen who were exposed to SM and in workers of SM manufacturing plants (10-12). Because of its alkylating and electrophilic properties, SM can alter chemical functional groups

* Corresponding author: Medical Genetics Department, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
E-mail: nazarabadim@mums.ac.ir

[ Downloaded from ijmcmed.org on 2026-11-07 ]

such as amines, carboxyls, S-H and O-H groups, and also primary phosphate groups (4). There are three distinct biochemical effects of SM: cytostaticity, mutagenicity, and cytotoxicity (4). Although considerable work has been focused on understanding the mechanisms of direct cellular injury mediate by SM exposure, relatively little is known about this phenomena. Several mechanisms have been proposed for the cytotoxicity of SM including; DNA damage, labilization of lysosomes and calcium mediated toxicity (6,7,13,14). SM like other mustards agents such as nitrogen mustard may possess teratogenic effects (15).

Craniofacial malformations are major human birth defects (16). with a worldwide frequency of 1 in 700 and substantial clinical impacts (17-19). Facial clefts represent the majority of these defects and can rise at any stage of development due to perturbation that alter the extracellular matrix as well as affect the patterning, migration, proliferation, and differentiation of cells (16). These deformities are believed to be caused by multifactorial inheritance of a threshold characteristic where several genes interact with environmental agents (4,20,21). An environmental component to clefting was recognized when Warkany et al. associated nutritional deficiencies with cleft palate (17,22). In addition, clefts may vary according to several influencing factors including time (4,23-28) and race (4,29-31). This report minimizes the time and race variability factors, thus focusing more precisely on environment and in particular SM-exposure.

In the event of an SM attack during war or a terrorist incident, the pregnant women might be one of the victims who survive the SM-exposure. However, the transplacentally exposed fetus may bear long term consequences.

Since comparatively little work has been conducted to assess the impact of SM on fetus teratogenicity, investigation of SM developmental toxicity should be considered. The aim of this study was to define the teratogenic effects of SM on cleft lip/palate on mouse embryo.

## Methods and Material

Reagents: Phenytoin (Dilantin®) was obtained from Parke Davis Company. SM (purity of 99.8%) was donated by Mashhad College of Pharmacy. Propylene Glycol was purchased from Merck Company (Germany). All other chemicals were of analytical grade and commercially available. All prepared solutions were stored at 4°C in the dark until administration.

Animals Care Statement: Both sexes of N. meri albino mice (mice south) were purchased from Razi Institute (Hesarak, Iran) and acclimatized for one week prior to treatment. Throughout the experiment, the mice were housed in a specific pathogen-free facility on corncob bedding with food and water ad libitum. The mice were randomly assigned to control and test groups. Seven mice were housed in each group.The gestational Day (GD) was defined as the date on which the vaginal plug was observed.

Animal Treatment: Pregnant females were IP dosed with 0.75 and 1.5mg of SM/kg of body weight. These doses were applied with regard to LD50 of 4.4mg/kg on GD 7; (32) the dose that will kill 50% of a group of animals under stated conditions. The control group was given the same volume of Phenytoin or Propylene Glycol. The schedule of administration is outlined in Table 1. On GD19, the mice were sacrificed by overdose of sodium thiopental. The gravid uterus of the pregnant mouse was harvested and weighed. The numbers and positions of the live or dead fetuses, as well as reabsorptions, were recorded. The live fetuses were weighed individually, gender determined and examined for external abnormalities.

Normal palatogenesis was assessed based on microscopic examination of the palate surface after an incision was made through the temporal-

[ Downloaded from ijmcmed.org on 2026-11-07 ]

mandibular joint. The cleft palate was scored if there was not fusion between the secondary palatal shelves (Fig.1).

These experiments were performed under the ethical guidance of Animal House of Ghaem Hospital, Mashhad University of Medical Sciences. Statistics: statistical analyses were plotted using Microsoft Excel. Data were analyzed by Chi-Square test followed by Fisher's Exact Test. The level of $p<0.05$ was considered significant.

**Table 1. IP injection Schedule of different drugs with definition of fetuses and the frequency of anomalies.**

| No. | Used material | Number of pregnant mice | Day of injection | Injection dose | Injection volume | Live Fetus | Dead Fetus | Resorbed Fetus | Mean Fetal Weight | CP |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | Phenytoin | 9 | G.D. 12 | 0.75 mg/kg | 0.1 ml | 77 | 0 | 0 | 1.33 ± 0.17 | 34 |
| **2** | _ | 8 | _ | _ | _ | 74 | 0 | 0 | 1.34 ± 0.18 | 0 |
| **3** | Propylene Glycol | 8 | G.D. 11 | 1.5 mg/Kg | 0.1 ml | 66 | 0 | 0 | 1.33 ± 0.23 | 0 |
| **4** | Propylene Glycol | 7 | G.D. 13 | 1.5 mg/Kg | 0.1 ml | 69 | 0 | 0 | 1.33 ± 0.24 | 0 |
| **5** | SM | 5 | G.D. 11 | 1.5 mg/Kg | 0.1 ml | 50 | 3 | 9 | 0.85 ± 0.39 | 28 |
| **6** | SM | 6 | G.D. 13 | 1.5 mg/Kg | 0.1 ml | 59 | 0 | 4 | 0.92 ± 0.5 | 21 |
| **7** | SM | 7 | G.D. 14 | 1.5 mg/Kg | 0.1 ml | 55 | 0 | 0 | 1.1 ± 0.48 | 0 |
| **8** | SM | 6 | G.D. 11 | 0.75 mg/Kg | 0.1 ml | 62 | 1 | 0 | 0.81 ± 0.13 | 12 |
| **9** | SM | 5 | G.D. 13 | 0.75 mg/Kg | 0.1 ml | 46 | 0 | 0 | 1.33 ± 0.2 | 0 |

GD; Gestational Day, CP; Cleft Palate.

Fig 1. Cleft Palate under Stereomicroscopy (left) and normal palate (right).

## Results

The results of pregnancy in SM treated groups are compared with control groups in 9 groups (Table 1). No indicative organ anomalies were observed in control negative and solution control groups. These results show that the incidence of cleft malformations in Phenytoin treated group was higher than control groups. In addition, the current data demonstrate that injection of 1.5 mg/kg in GD 11 significantly increase the incidence of cleft anomalies in comparison with the control group ($p<0.001$), but no obvious teratogenic activity of SM could be observed on GD14. The rate of anomalies was also slightly higher in GD11 compared with GD13. On the other hand, the incidence of malformations were more prominent in the 1.5 mg/kg than 0.75 mg/kg ($p=0.01$).

## Discussion

Sulfur mustard (SM), commonly known as mustard gas, is an alkylating agent which was widely used as a chemical warfare during Gulf war



[ Downloaded from ijmcmed.org on 2026-11-07 ]

against soldiers and civilians (1). The previous reports have demonstrated the ability of this class of compounds to cause adverse effects (15). However, very few correlations have been established between SM exposure and congenital cleft lip/palate deformity. Similar experimental works were carried out on its analog; Nitrogen Mustard, which revealed that it can lead to different malformations such as: cleft palate, functional and structural anomalies and some growth defects (15). These data demonstrate that the teratogenic effects of SM are restrictively under the influence of the gestation time (during organogenesis) and the threshold dose. The critical period of the different organs may interfere and therefore, exposure to a single teratogen in a specific day may cause several anomalies. On the other hand, the organ specific critical period may take several days long and the sensitivity of organs to teratogens can vary greatly in different periods. Therefore, a specific dose of a teratogen in different days may cause different anomalies and increases the rate of malformations. Teratogens can interfere with cleft morphogenesis through different pathogenetic pathways such as: mutation, cytotoxicity and enzymatic changes. A number of mechanisms have been proposed for these pathways including; DNA damage, labialization of lysosomes and calcium mediated toxicity (6). The emphasis on teratogenic influences has not led to elucidation of pathogenetic pathways, so the potential mechanisms of induction of cleft palate defects by SM are considered to be important areas of research in future.

In a similar study done by McNamara et al. (33) pregnant rats were exposed to SM by gastric intubation in different doses. It was claimed that, no evidence of teratogenicity was observed. Such a discrepancy results could be explained by different routes of drug administration and doses which had been used.

This study indicated that within a population of pregnant mice, exposure to SM was directly correlated with increased risk of congenital cleft malformations. Therefore, the transplacentally exposed fetus which may survive SM attack can bear long term consequences. Our data demonstrate that the teratogenic effects of SM are restrictively under the influence of the time of gestation (during organogenesis), as well as the threshold dose. Considering the destructive effects of mustard gas on different organs, the logical question that one may ask is why despite the conventional laws that prohibit the use of these weapons, nevertheless, it has been recently used against the innocent human beings.

## Acknowledgements

We wish to express our appreciation to the Vice President for Research of Mashhad University of Medical Sciences for the finanical support and to Dr. Arghami for the statistical analysis.

## References

1. Bijani Kh, Moghadamnia AA. Long-term effects of chemical weapons on respiratory tract in Iraq-Iran war victims living in Babol (North of Iran). Ecotoxicol Environ Saf 2002;53:422-4.

2. Balali M. Clinical and laboratory finding in Iranian fighters with chemical gas poisoning. First World congress on Biological and Chemical Warfare Agents; 1989 May 21-23; Ghent, Belgium. 1989;254-9.

3. Compton JF. Chemical and toxicological properties. In: Compton JF, editor. Military Chemical and Biological Agents. Caldwell; 1999. p. 5-17.

4. Taher AA. Cleft Lip and Palate in Tehran. Cleft Palate Craniofac J 1992;29:15-6.

5. WHO. Health aspect of biological and chemical weapons. URL: http://www.who.int/csr/delibepidemics/biochem1stenglish/en/index.html; WHO; 1970.

6. Lakshmana Rao PV, Vijayaraghavan R, Bhaskar AS. Sulfur mustard induced DNA damage in mice after dermal and inhalation exposure. Toxicology 1999;139:39-51.

7. Ludlum DB, Papirmeister B. DNA modification by sulfur mustards and nitrosoureas and repair of these lesions. Basic Life Sci 1986;38:119-25.



[ Downloaded from ijmcmed.org on 2026-11-07 ]

8. Rall DP, Pechura CM. Effects on health of mustard gas. Nature 1993;366:398-9.

9. Freitag L, Firusian N, Stamatis G, et al. The role of bronchoscopy in pulmonary complications due to mustard gas inhalation. Chest 1991;100:1436-41.

10. Iwaszkiewicz J. Burns of the upper respiratory tract caused by mustard gas. Pol Med J 1966;5:706-9.

11. Aasted A, Darre E, Wulf HC. Mustard gas: clinical toxicological and mutagenic aspects based on modern experience. Ann Plast Surg 1987;19:330-3.

12. Easton DF, Peto J, Doll R. Cancers of the respiratory tract in mustard gas workers. Br J Ind Med 1988;45:652-9.

13. Somani SM, Babu SR. Toxicodynamics of sulfur mustard. Int J Clin Pharmacol Ther Toxicol 1989;27:419-35.

14. Ribeiro PL, Mitra RS, Bernstein IA. Assessment of the role of DNA damage and repair in the survival of primary cultures of rat cutaneous keratinocytes exposed to bis (2-chloroethyl) sulfide. Toxicol Appl Pharmacol 1991;111:342-51.

15. Wormser U, Izrael M, Eddy A, et al. A chick model for the mechanisms of mustard gas neurobehavioral teratogenicity. Neurotoxicol Teratol 2005;27:65-71.

16. Young DL, Schneider RA, Hu D, et al. Genetic and teratogenic approaches to craniofacial development. Crit Rev Oral Biol Med 2000;11:304-17.

17. Murray JC. Gene/environment causes of cleft lip and/or palate. Clin Genet 2002;61:248-56.

18. Strauss RP. The organization and delivery of craniofacial health services: the state of the art. Cleft Palate Craniofac J 1999;36:189-95.

19. Vanderas AP. Incidence of cleft lip, cleft palate, and cleft lip and palate among races: a review. Cleft Palate J 1987;24:216-25.

20. Fraser FC, Calnan JS. Cleft lip and palate seasonal incidence, birth weight, birth rank, sex, site, associated malformation, and prenatal age. A statistical survey. Arch Dis Child 1961;36:420-3.

21. Dirillin CM, Ingram TTS, Wilkinson EM. The causes and natural history of cleft lip and palate. Edinburgh: Churchill, Livingstone; 1966.

22. Warkany J, Nelson RC, Schraffenberger E. Congenital malformations induced in rats by maternal nutritional deficiency. Am J Dis Child 1943;53:309-17.

23. Dallare L, Melancon LSB, Potier M, et al. Date of conception and neural tube defect. Clin Genet 1984;26:304-7.

24. Edward JH. Seasonal prevalence of congenital disease in Birmingham. Ann Hum Genet 1961;25:89-93.

25. Elwood JM. Seasonal variations in anencephalus in Canada. Br J Prev Soc Med 1975;29:22–6.

26. Fugino H, Tanaka K, Sanuly Y. Genetic study of cleft lip and cleft palate based on 2828 Japanese cases. Kyushu J Med Sci 1963;14:317-31.

27. Abbas AY Taher. Cleft Lip and Palate in Tehran. Cleft Palate Craniofac J 1992;29:15-6.

28. Saxen I, Lahti A. Cleft lip and palate in Finland: incidence, secular, seasonal and geographic variations. Teratology 1974;9:217-23.

29. Amartunga AND, Chanadrasekera A. Incidence of cleft lip and palate in Sri Lanka. J Oral Maxillofac Surg 1989;47:559-61.

30. Chapman CJ. Ethnic difference in the prevalence of cleft lip and/or cleft palate in Aukland. N Z Med J 1983;96:327-9.

31. Chung CS, Myrianthopulous NC. Racial and prenatal factor in major congenital malformations. Am J Hum Genet 1968;20:44–60.

32. Anslow WP, Karnofsky DA, Jager BV, et al. The intravenous, subcutaneous and cutaneous toxicity of bis(f3-chloroethyl)sulfide (Mustard Gas) and of various derivaties. J Pharmacol Exp Ther 1948;93:1-9.

33. McNamara BP, Owens EJ, Christensen MK, et al. Toxicological basis for controlling levels of mustard in the environment. Edgewood Arsenal: Aberdeen Proving Grounds; 1975.

[ Downloaded from ijmcmed.org on 2026-11-07 ]