Fetal Exposure to Endocrine Disrupters during Human Pregnancy in Japan

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Thank you, chairman. I am very honored to be here to present our paper in this symposium. I would like to talk about the status of maternal and fetal exposure to environmental disrupters. In our presentation, however, very limited data were obtained, but we think we have very strongly suggestive and important results.

I am demonstrating the first part of our results, and Dr. Kurosawa is presenting the second part of our results. Today's topics: first of all I would like to talk about the hypospadias prevalence in Japan and secondly I would like to talk about the fetal and maternal exposure to endocrine disrupters. Finally, Dr. Kurosawa is presenting about hypospadias and bisphenol A.

Here you can see a lot of teratogenic factors surrounding us, such as infectious agents, viruses, other infectious agents, drugs such as thalidomide or drugs, the surrounding the radiation and electromagnetic fields, and also endocrine disrupters are one of the teratogenic factors.

Here are the endocrine disrupters related to the reproductive abnormalities for males: sperm count dysfunction or undescended testes or hypospadias; also the testicular tumors are suspected to be partly due to endocrine disrupters. Also, the female reproductive abnormalities are shown here. The study was aimed to evaluate the condition of fetal and maternal exposure to endocrine disrupters, namely bisphenol A in human pregnancy.

The background of this study: I would like to just talk about that a little bit, about our study's background. According to the Japan Birth Defect Registry, some malformations including hypospadias are showing an increasing prevalence trend. Here is the hypospadias prevalence in Japan. Here is from 1972 until 2001. Here is the trend figure of hypospadias in our country. The rate is per 10,000 births, and this data is collected from the Japan Birth Defect Registry.

The Japan Birth Defect Registry is located in our institution and this program is participating in the International Clearinghouse for Birth Defects Monitoring Systems. This is the Japan Birth Defects Registry district map. In 9 divided districts, we are collecting the data from these districts, and the headquarters are located in Yokohama close to Tokyo. Hiroshima is here.

I would like to explain the Japan Birth Defects Registry a little more. This registry is a nationwide hospital-based monitoring system since 1972. This registry is covering about 10% of all births in Japan. After 22 gestational weeks until 7 days postpartum, more than 150 markers are registered.

From the International Clearinghouse for Birth Defects Monitoring Systems, hypospadias increased in these countries. No changes are recorded in these countries; particularly in England quite recently no changes were recorded there. Anyway, Japan is one of the increasing countries. But there are no countries where hypospadias decreased.

In our country, the prevalence rate of hypospadias is 3-4/10,000; not such a high rate as compared to the western countries, and around 500 affected infants were born every year. So we have to consider about the etiology of the hypospadias, like these etiological factors.

Epidemiologically, there is an increasing trend in hypospadias; on the other hand from etiological factors endocrine disrupters are suspected as one of the causes. So the question has been raised: what kind of relationship between the endocrine disrupters and human congenital abnormalities such as hypospadias?

Therefore, we have started to evaluate the fetal exposure to endocrine disrupters in human pregnancy. We chose bisphenol A. This is one of the weak environmental estrogens, and bisphenol A is widely used and exposed in our daily life in metal food cans, food containers, compact discs, and glassware.

The blood samples were collected from first trimester, at 36 gestational weeks, during delivery and also from umbilical cord blood. Also, we collected the clinical information; the samples were assayed by ELISA system.

We collected 1 ml of serum from each individual and the ELISA for immunoreactive bisphenol A using the anti-BPA polyclonal antibodies. The assay system has been generated by Otsuka Assay Laboratories and Yanaihara Institute. This data has good correlation to HPLC data.

This is the data of our study. Among the pregnant women, from 815 pregnant women, 790 women were detected, about more than 97%. In other words, more than 97% of the women were contaminated. The average of bisphenol A was 0.4 ng/ml.

On the other hand, with umbilical cord blood, 100% of the samples were detected, from 398 babies. The data is shown here 1.37 ng/ml. You can see in the figure, here is the pregnant women's level about 0.4 ng/ml. On the other hand, umbilical cord blood is much higher than the pregnant mother's level.

We also looked at the pregnant women's urine and blood. These data are quite similar, not so different.

Along with gestational weeks, we checked the bisphenol A levels in the pregnant women, but there were no remarkable changes along with the gestational weeks. From the 6th-9th week until the 36th week, there are no remarkable changes along with gestation.

When looking for the relationship between the maternal BPA and the cord blood BPA value, there is no significant relationship between these values. But if you divide the 2 groups when you collected the data into a group with less than 0.45 ng/ml BPA maternal blood and a group with more than 0.45 ng/ml maternal blood, there is a significant difference in umbilical cord blood BPA. So higher maternal levels showed a higher cord blood BPA level.

Why is the cord blood BPA high? So far we have no clear explanation for this phenomenon, but we can speculate on these mechanisms. One of the mechanisms is accumulation: somehow, some kind of mechanism is there for accumulation of BPA and some special fetal metabolism is there, or active transport system. But we have no evidence at the moment.

The question is whether the high level of BPA is harmful to the fetus. Using animal experiments, Dr. vom Saal and other investigators are reporting these data, but in human beings there is no data at the moment.

In our study, a very few number of the birth defects cases were collected. In this data, the mother's BPA level showed 0.65 ng/ml which is a little higher than a normal control.

Conclusions: hypospadias is gradually increasing in Japan. The reason for this trend is not clear at the moment. Higher levels of BPA in the umbilical cord blood were shown as compared to maternal BPA levels. For ecotoxicological risk assessment, further studies including epidemiological and embryopathological approaches are required.

Acknowledgments are shown here. Thank you for your attention.

Q&A

Toppari: Thank you for this interesting data. Now we are in time for a break, but we may have 1 or 2 short questions. There is a lot of interest in this now, but try to be short, please.

Nishikawa: My name is Nishikawa, and I'm with Mitsubishi Chemical Corp. You just made a comparison of the figures 0.82 and 0.40 in pregnancy. The concentration of BPA in the blood of mothers giving birth to babies with hypospadias was 0.82. It is my understanding that the mothers you are talking about are mothers that gave birth several years prior to this measurement. Is this correct?

Kurosawa: The age range of the children is 1 through 16, so the range would be 1 to 16 years after giving birth.

Nishikawa: The figure for pregnant women therefore would be 0.40, but is not a control group. If it were, I think it would be appropriate to compare with females who gave birth several years ago or with normal females who have never given birth. What about this figure?

Kurosawa: Just as you pointed out, it is difficult to establish control group in an experiment involving so many targets. Getting accurate control group is a theme we have to deal with. As you pointed out, it would be an overstatement to say that the data was obtained under precise control.

Nishikawa: Probably an overstatement. What about the difference between pregnant women and normal women who are not pregnant? Do you have data for this?

Kurosawa: No, I don't. The data concerns mothers of ordinary age who are not pregnant, mothers of same age as this study and those who have at least born a child. No, I don't have any data.

Nishikawa: Although you don't have the data, several reports have been published. Dr. Tsutsumi also reported 2 ng/ml on the first day of the symposium. If this were used as a control, it suggests that BPA concentration is oppositely low, and since different methods of analysis are being used, a sweeping comparison cannot be made. I however thought it was strange the concentration of BPA is higher in women with hypospadias than so-called normal women, or women who are not pregnant.

Kurosawa: Thank you very much.

Iwamoto: I have three questions.

First of all, you used polyclonal antibodies when measuring BPA, but this is extremely dangerous. Other matter could adhere to them all. This has been confirmed with HPLC. If retention time differs, strange results will be obtained. So my first question is what about performing this experiment again using monoclonal antibodies?

Another one is, as Dr, Guillette said a little while ago, the conclusion from the beginning…was pesticides. Then there is the question of exposure or no exposure. I think this method is very dangerous for epidemiology. The reason for this is perhaps because substance such as dioxins and hydroxy PCB should be measured. This should be done completely. According to those involved in this research, at tens of picograms, as Dr. Tanabe said a little while ago, concentration in Japan is quite high. I think such substances considerably have an effect.

Finally, concerning the age of the mothers, this often occurs when the mother is too young. Have you analyzed this?

Hirahara: Like you just pointed out, there is some question concerning the measurement method. We however used this assay system because we will never be able to move ahead if we can't overcome the problem of dealing with a large volume of specimens with a small amount of blood. As for pilot data, we have started using favorable relationship with HPLC as a prerequisite. I think

processing of data is possible for this.

I think various other problems will emerge in the future. We have by no means established a method of control or drawn a conclusion. There are many factors that must be analyzed as data for a midterm report.

Toppari: We have to stop the discussion here because we want to keep the timetable. We want to keep the timetable please, because we want to have a break before the discussion. Thanks for the lively discussion and thanks for the presentation. We hope that there will be time during the discussion for everybody to participate in that. Thank you.