Bisphenol A in Saliva and Urine Related to Placement of Dental Composites (NCT02575118)

RESEARCH PROTOCOL (Rev 22.06.16)

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Concentration of bisphenol A in saliva and urine after treatment with polymer-based dental restorative materials

Project summary

Bisphenol A (BPA) is an estrogen-mimicking substance, which even at low concentrations, has been shown to cause abnormalities in sex development and lead to reproductive damage in fish and animals. In population-based studies, BPA has been detected in concentrations that indicate levels that could cause health effects.

The majority of dental plastic filling materials contain methacrylates which are synthesized from, among other substances, BPA. It has not been sufficiently investigated whether BPA is released in vivo from polymer-based dental fillings.

The purpose of the project is to gain knowledge about the BPA concentration in saliva and urine, and whether it is affected by newly placed polymer-based fillings. We will measure and compare the saliva and urine concentrations of BPA before and after filling therapy in individuals who receive their first composite filling.

Project Manager

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Project staff

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Responsible research institution

Uni Research AS will be responsible research institution. Owners of data will be the Dental Health Service in Hordaland and Uni Research AS.

Collaborators

The project is organized as a collaborative project between the Dental Health Service, Hordaland County and the Dental Biomaterials Adverse Reaction Group (Uni Research Health).

The Public Dental Health Service, Hordaland County, contributes with

• Access to project participants

• Access to journal archive. Working hours to perform clinical examination, sampling and filling therapy on the subjects, as well as design of necessary information and questionnaire (TK-Vest Hordaland)

• Dental office

The Dental Biomaterials Adverse Reaction Group (Uni Research Health) contributes with

• Working time

• Guidance

Background

Bisphenol A (CAS No. 80-05-7) is a chemical substance that has been shown to be weakly hormone-mimicking, so-called estrogen-mimicking. Excess estrogens can lead to reduced development of male sex characteristics and reduced fertility.

The concentration of bisphenol A measured in human blood and urine cannot be explained by previously described routes of exposure and known half-lives [1]. This indicates a higher intake of bisphenol A than what has been calculated so far and in addition, bioaccumulation of bisphenol A after exposure. A study of bisphenol A concentrations in urine in relation to food intake [2] confirms this. This study showed that concentrations of bisphenol A in urine decreased more slowly than might be expected based on previously calculated half-lives. The authors conclude that there is a significant "nonfood exposure" and / or bioaccumulation.

The majority of polymer-based dental filling materials contain methacrylates which are produced from, among other substances, bisphenol A. Although bisphenol A is not added as an ingredient, contaminated raw materials may contain low concentrations.

It has been shown that treatment with some fissure sealing materials containing bis-DMA (bisphenol A dimethacrylate) may result in a short-term exposure to bisphenol A immediately after treatment [3].

Another study [4] has observed that filling therapy with plastic-based filling material is associated with a higher concentration of bisphenol A in saliva and urine immediately after treatment.

Differences in sampling methods, examined materials, follow-up time and analysis methods and detection levels give variation in the results.

Results from a recent study [5] showed that it could not be ruled out that individuals with plastic-based dental fillings have a higher concentration of bisphenol A in saliva compared to individuals without plastic-based dental fillings. More research on the subject is warranted.

Aim

The aim of the project is to gain knowledge about the concentration of bisphenol A in saliva and urine in individuals before and after treatment with a polymer-based dental restorative material.

We want to test a hypothesis (H_o) that the bisphenol A concentration in saliva and urine is unchanged or lower after filling therapy with a polymer-based dental restorative material. The alternative hypothesis (H_1) is that bisphenol A concentration in saliva and urine increases after treatment.

Materials and methods

Study design

20 healthy and voluntary individuals (10 women and 10 men) who need a minimum of two tooth surfaces treated with a polymer-based dental restorative material are recruited from dental clinics in Sentrum Dental Health District, Hordaland County and from the Student Association's dental clinic in Bergen.

Inclusion criteria:

Need for filling therapy that involves at least two surfaces with a polymer-based dental restorative material.
Healthy patient (No known chronic diseases)
Age: 16 - 40 years
Written informed consent

Exclusion criteria:

Substance abuse
Smoking
Use of ('snus') snuff
Treated with polymer-based dental restorative material the last 3 months
Removable prosthetic dentures or orthodontic appliances
Use of dental splint
Work at a dental clinic

• Informed consent will be obtained according to current rules.

• All subjects answer questions related to dietary habits and living habits. (See attached questionnaires 1, 2 and 3)

Recruitment

Patients attend an ordinary examination at their regular dental clinic. If the dentist/dental hygienist considers that the patient needs filling therapy with polymer-based dental restorative material and is otherwise suitable for the study (inclusion/exclusion criteria), the patient receives oral information about the project and is asked if he/she wishes to participate. If the patient agrees, the name and contact information are entered on a recruitment list (see appendix). At the same time, the patient is given an information letter with a thorough description of the study and a consent form (see appendix). It will be emphasized that participation is voluntary. The patient will have the opportunity to read through the information letter at home before he/she is contacted and possibly given an appointment with the project manager.

Day 1 with project manager

• Project participant meets in the morning with written consent.

• The participant must be fasting, i.e. not eating or drinking for the last 10 hours. Only tap water is allowed. The bladder must be emptied of morning urine at home, preferably as soon as possible after waking up. The time of emptying is noted by the participant. Avoid lip balm/lipstick.

• Dental status is mapped according to ordinary clinical procedure. If polymer-based dental restorative material is detected in the oral cavity, the individual must be excluded.

• Project participant answers questions from questionnaire 1.

• Saliva and urine samples are collected according to specified methods (See appendix).

Filling therapy is performed according to the ordinary procedure.

Day 2 with project manager

• The patient meets fasting, i.e. has avoided food and drink for the last 10 hours. Only tap water is allowed. The bladder must be emptied of morning urine at home, preferably as soon as possible after waking up. The time of emptying is noted by the participant. Avoid lip balm/lipstick.

• Answers questionnaire 2.

Saliva and urine samples are collected from the subjects according to specified methods

Day 8 with project manager

• The patient meets fasting, i.e. has avoided food and drink for the last 10 hours. Only tap water is allowed. The bladder must be emptied of morning urine at home, preferably as soon as possible after waking up. The time of emptying is noted by the participant. Avoid lip balm / pins.

• Answers questionnaire 3.

Saliva and urine samples are collected from the subjects according to specified methods

Work protocols are stored at the clinic (see appendix).

Filling materials

As an adhesive Optibond FL will be used and as a filling material Tetric Evo Ceram (capsules from Ivoclar Vivadent AB).

The materials are chosen because they are used routinely at the relevant dental clinic.

Saliva samples and urine samples

Saliva and urine samples are collected from the subjects before and after filling therapy according to the specified method.

	Just before treatment	Just after treatment	1 hour after treatment	24 hours after treatment	1 week after treatment
Saliva test no.	0	1	2	3	4
Urine sample no.	0		1	2	3

Before sampling, the sampling chain is checked so that the risk of contamination is minimized. The samples are immediately placed in a refrigerator and frozen as soon as possible at -80 ° C before analysis.

Do not use equipment that can cause contamination from plastic products/bisphenol A-containing products. The effect of the curing lamp is checked and documented weekly for as long as the project lasts. Previous filling therapy and points are registered in a separate diagram.

Filling therapy

• Detect current tooth surfaces that need filling

• Filling therapy according to normal clinical routines

• Any rubber-dam use is registered

• Estimate the amount of composite/surface area (Weigh the composite ampoules before and after filling therapy).

• Type of curing lamp and curing time are registered.

Analyzes

Analysis of bisphenol A in saliva and urine is performed as a commissioned analysis at the Department of Occupational and Environmental Medicine (AMM), Lund University, Sweden. A method of liquid chromatography (LC) and mass spectrometry (MS) (LC-MS) which we have used in a previous project [5] will be used.

Possible advantages and disadvantages for the participants

Participation in the project will mean that the patient must give five saliva samples (just before filling therapy, just after filling therapy, after 1 hour, after 24 hours and after 1 week) and four urine samples, which entails extra visits to the dental clinic. As a small appreciation, all participants will be given two cinema tickets and two lottery tickets (“Flax”) after passing the last urine test. Paying patients (persons over 18 years of age) will not be charged financially for the treatment in question.

Outcome

Concentration of bisphenol A in saliva and urine.

Time plan

August 2014
Assessment by local Research Ethics Committee

September 2015 - April 2017
Recruitment of and filling therapy on project participants

May - June 2017
The saliva and urine samples are analyzed

August - October 2017
Data analyses

October - December 2017
Publication of poster and manuscript

Statistical analysis

The primary outcome measure is the concentration of bisphenol A in saliva. Data from an unpublished study [5] showed that the concentration of bisphenol A in saliva is very low and in many samples below the detection limit (0.1 ng / ml saliva). Therefore, non-parametric tests are primarily used for the statistical analysis of the saliva data. Values below the detection limit are given ½ the value of the detection limit in the statistical analyzes.

Concentration of bisphenol A in urine is a secondary outcome measure. Concentrations of bisphenol A in urine in individuals without plastic fillings are probably above the detection limit in most cases, and data are probably not normally distributed. Data on bisphenol A in urine are therefore primarily analyzed also with non-parametric tests (Friedman test and Wilcoxon Signed Rank Test for pairwise testing of data).

For analysis of observations before treatment (t_0), respectively after treatment ($t_{10\ min} = t_{4\text{-}7\ hrs} = t_{24\ hrs} = t_{7\ days}$) Friedman test is used, which tests the hypothesis (H_0), that the values at all times are equal (i.e. $t_0 = t_{10\ min} = t_{4\text{-}7\ hrs} = t_{24\ hrs} = t_{7\ days}$).

For the analysis of observations divided into the groups "<detection limit" and "≥ detection limit", respectively, before and after treatment, McNemar's test is used, which compares observations from two times at a time. SPSS will be used for the statistical analyzes (Version 22).

Power / sample size

Reliable data that can be used to calculate sample size is missing. Therefore, the following assumptions are made for saliva data (see Figure 1):

1. The proportion of individuals with values below the detection limit before treatment and above (or equal to) the detection limit after treatment is 0.7 (i.e. 70%).

2. The proportion of individuals with values above (or equal to) the detection limit before treatment and below the detection limit after treatment is 0.1 (i.e. 10%).

3. The proportion of persons who show no change, i.e. they have values below the detection limit both before and after treatment 0.1 (10%) or values above (or equal to) the detection limit both before and after treatment 0.1 (10%).

		Before treatment	
		< d.l.	≥ d.l.
After treatment	< d.l.	0,1	0,1
	≥ d.l.	0,7	0,1

Figure 1. Proportions with values below and above the detection limit (d.l.) before and after treatment.

Power calculation

If a one-sided test (Mc Nemar test of paired proportions) is used, a study with 10 subjects then has a power of 77.5% to be able to detect a significant difference before and after treatment with significance level alpha set to 0.05 . If the number of subjects increases to 20, a power of 96% is obtained (Figure 2).

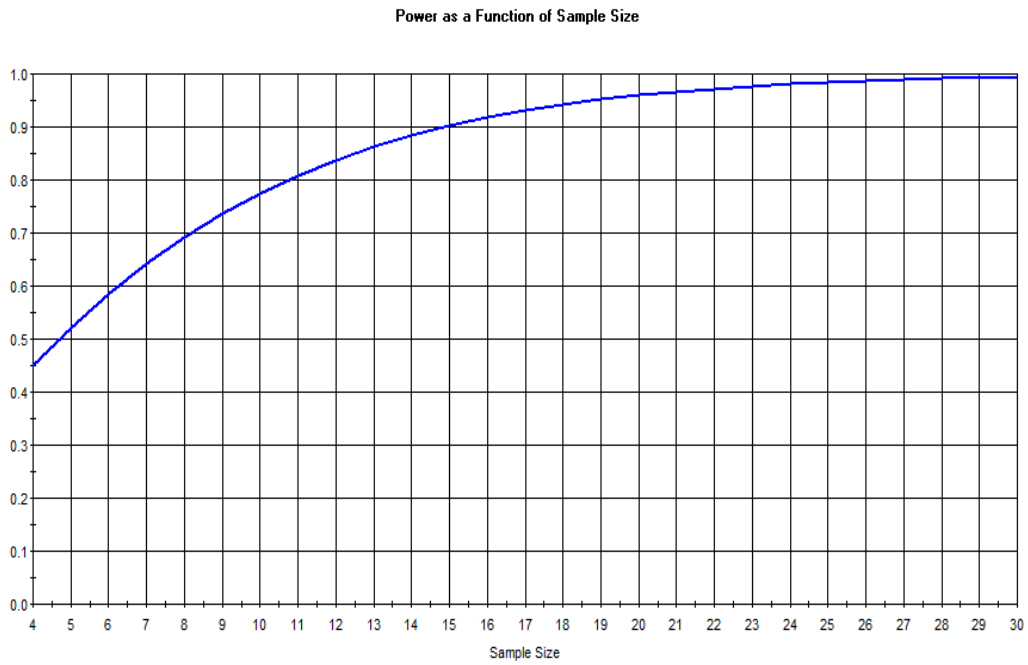

Figure 2. Test strength (power) as a function of sample size (one-sided test). (From SPSS Sample Power; Mc Nemar test of paired proportions).

Handling of possible adverse events

As we see it, there is no particular risk that the project will lead to an increased risk of adverse events. Should complications occur, these will be handled according to the dental clinic's usual procedures.

Assessment of research ethics

The project will be assessed by the Regional Committee for Medical and Health Research Ethics.

In our opinion, the benefits of carrying out the project are significantly greater than the disadvantages. Participation in the project does not pose a significant risk the subjects, beyond what ordinary dental treatment may entail.

Previous studies have shown that bisphenol A can be detected in saliva and urine. The source of this is unclear and more knowledge is needed. Increased knowledge of potential risks with dental filling materials is important for choosing safe dental restorative materials.

If it turns out that bisphenol A cannot be detected in saliva and urine after filling therapy, the probability that dental filling materials are a source of exposure to bisphenol A is low.

The participants are made aware that participation is voluntary and that they can withdraw from the project at any time.

All subjects must sign informed consent, approving that the samples can be used for analysis. Patient data will be pseudonymized and processed properly after approval.

All information and samples from the participants will be processed without name and date of birth. Each participant will be linked to the project by a code corresponding to a list of names. Only personnel directly connected to the project have access to the name list. It will not be possible to identify the participants in the project when the results are published.

After the last urine test, the participants will be given two cinema tickets and two lottery tickets (“Flax”) as an appreciation for the participation. All patients, including people over the age of 18, will receive the actual dental filling free of charge. In our opinion, the total value of this compensation does not exceed what can be considered reasonable for participation.

Publication - dissemination plan

The project started in the autumn of 2015 and is planned to be completed by the end of 2017. This means that there will be a poster for presentation at a scientific meeting and a manuscript for submission to a scientific journal.

Budget (omitted)

Significance

The results could provide important information to assess whether filling therapy with polymer-based dental restorative materials is associated with increased concentration of bisphenol A in saliva. We also have an opportunity to look at the concentrations in urine.

If the results from the study show that there is an association between exposure to dental polymer-based dental restorative materials and bisphenol A in biological media, this motivates follow-up studies.

References

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