Fragments od bacterial DNA presence in hemodialysis patients’ blood-preliminary report

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ABSTRACT

Cardiovascular disease (CVD) accounts for most of morbidity and mortality in patients with end-stage renal disease treated by chronic hemodialysis. The idea that inflammation state present in uremia plays an important role in the development of atherosclerosis has enjoyed much attention. We have investigated therefore possible presence of bacterial DNA in the blood of chronically hemodialyzed patients. There were retrospectively studies of 59 patients without any signs of active infection, who had been undergoing intermittent HD. In 54 among 59 investigated DNA blood samples from HD patients we were able to detect a DNA fragment about 790 bp in length, consistent with the presence of 16sRNA bacterial DNA. Bacterial DNA could not be detected in any of the samples from control subjects. We were not able to detect bacterial DNA in any of 9 investigated dialysate samples although in 4 blood samples of these patients bacterial DNA encoding ribosomal RNA was observed. Bacterial DNA is detectable in blood of HD patients. This might be one of the inflammatory stimuli.
INTRODUCTION

Cardiovascular disease (CVD) accounts for most of morbidity and mortality in patients with end-stage renal disease treated by chronic hemodialysis (1). Even proper management of those patients, which helps to prevent hypertension, does not fully answer the problem (2). Traditional risk factors do not adequately explain excess CVD observed in ESRD patients, and paradoxically some of them are inversely related to prevalence of CVD a phenomenon called “reverse epidemiology” (3). Thus the idea that inflammation state present in uremia plays an important role in the development of atherosclerosis has enjoyed much attention (4,5). This notion has found support in reports of association between markers of inflammation (CRP, pro-inflammatory cytokines) and increased mortality. It was found that 30-60% of HD patients have evidence of activation of inflammation (6). The stimulus or stimuli for such a response were not found however. Several of them were proposed such as reduced renal clearance of cytokines, accumulation of AGE’s, occult inflammatory processes or infections, bioincompatibility of dialysis membranes and exposure to endotoxins and unspecified pro-inflammatory substances present in dialysis fluid (7,8). Schindler et al. have reported on the presence of short bacterial fragments in dialysate and induction of cytokines and TLR9 by them (9). These fragments were of sufficient small size to pass through dialyzer membrane. We have investigated therefore possible presence of bacterial DNA in the blood of chronically hemodialyzed patients.

MATERIAL AND METHODS

There were retrospectively studies of 59 patients without any signs of active infection, who had been undergoing intermittent HD (tab. 1).

DNA samples isolated from whole blood, which were stored at -20C as a part of an ongoing prospective study on the CRP gene polymorphism effect on the CVD prevalence, were randomly selected. DNA samples was also isolated from dialysate of 9 different dialysis machines.100ml of dialysate and 5 ml of whole blood anticoagulated with 0.48% citric acid, 1.32% sodium citrate 1.47% glucose were withdrawn before HD session. Microbiological purity of water and dialysate was monitored according to Polish Pharmacopoeia VI regulations. Control subjects - 109 healthy volunteers (hospital staff) donated blood for DNA isolation.

DNA was isolated using Macherey-Nagel kit according to the manufacturer procedure after pretreatment of samples by three enzymatic digestions (Fig.1)

PCR was performed with universal primers for 16sRNA bacterial gene: forward 5’ AGT TTG ATC CTG GCT CAG and reverse 5’ GAA CTA CCA GGG TAT CTA AT (Oligo Poland), 5 ng of DNA, Fast Start Taq DNA polymerase Kit, GC rich (Roche) and PCR Anty-Inhibitor (DNA Gdańsk, Gdańsk, Poland), in final volume of 25 µl.

RESULTS

In 54 among 59 investigated DNA blood samples from HD patients we were able to detect a DNA fragment about 790 bp in length, consistent with the presence of 16sRNA bacterial DNA. Observed bands were differing slightly in length and density suggesting different bacterial species as a DNA source and different concentrations in blood (Fig. 3). Bacterial DNA could not be detected in any of the samples from control subjects. We were not able to detect bacterial DNA in any of 9 investigated dialysate samples although in 4 blood samples of these patients bacterial DNA encoding ribosomal RNA was observed.

CONCLUSION

Bacterial DNA is detectable in blood of HD patients. This might be one of the inflammatory stimuli. There are several possible sources of bacterial DNA in blood of HD patients: passing of oligonucleotides through dialyzer membrane, endogenous sources (from atherotic plaques, paradontium, nasal sinuses, veins wall, aortic wall, dialysis fistula) or repeated introduction of bacteria into bloodstream during puncturing of dialysis fistula. Normal skin is colonized predominantly by Gram-positive bacteria, like Staphylococcus spp., Micrococcus spp., and coryneforms, whereas only Acinetobacter spp. are only Gram-negative bacteria regularly colonizing normal skin. External factors, like topical antiseptics, however may promote skin colonization by Gram-negative bacteria. Due to preliminary nature of this work we were not able to ascertain neither the source DNA nor bacterial
species involved.

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Fig. 1. DNA isolation
Fig. 2. ELEKTROPHORESIS
Fig. 3. Bacterial 16S rRNA gene

UNIVERSAL - HIGHLY CONSERVED REGION OF DNA

FIG. 1. DNA ISOLATION

1. muramidase (SIGMA), incubation for 3h at 37°C, pH 4.8
2. lysozyme (Roche), incubation for 1h at 37°C, pH 7.0
3. proteinase K added in appropriate buffer from NucleoSpin®

FIG. 2. ELEKTROPHORESIS

FIG. 3. BACTERIAL 16S RRNA GENE UNIVERSAL - HIGHLY CONSERVED REGION OF DNA

Broad-range bacterial PCR is based on the use of primers that recognize conserved sequences of bacterial chromosomal genes encoding ribosomal RNA (rRNA). The resulting amplified rRNA sequences also include variable regions that provide an alternative approach for identifying theoretically all bacterial species, including those that cannot be cultivated by classical methods.
### TAB. 1. CHARACTERISTIC OF PATIENT

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>Male gender (%)</td>
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<tr>
<td>Age of years</td>
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<tr>
<td>Median value of CRP (ng/ml)</td>
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<tr>
<td>Median period of HD (months)</td>
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<td>Frequency per week</td>
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<td>Length of HD (h)</td>
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<tr>
<td>Fistulae</td>
<td>native arteriovenous, the double-needle technique</td>
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<td>Machines</td>
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<td>Dialysers</td>
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<tr>
<td>Dialysate</td>
<td>bicarbonate-buffered</td>
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<td>The enrolment criteria</td>
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<tr>
<td>Patients excluded</td>
<td>diabetes, SLE, malignancy, HIV, severe liver disease, colitis ulcerosa, Crohn disease</td>
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### BIBLIOGRAPHY