Fecal *Bifidobacterium* Levels in Elderly Nursing Home Patients—Are Levels as Expected?

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We assessed the fecal bifidobacteria concentrations of 21 nursing home subjects prior to death and 21 age- and sex-matched controls. Bifidobacterial levels, determined by molecular methods, were in the range of those usually found in adults. Total fecal bifidobacterial concentrations determined by fluorescent in situ hybridization and quantitative real-time PCR tended to be lower, although not significantly, in subjects who subsequently died than in age- and sex-matched controls.

Key words: *Bifidobacterium*; microbiota; elderly

INTRODUCTION

Intestinal colonization and microbiota succession initiate at birth and continue until the end of life. According to traditional culture-based methods the human intestinal microbiota changes in composition in old age with significant decreases in bifidobacteria *(3, 8, 9, 15, 17)*. Some support for this observation is also offered by recent studies using molecular methods *(10)*, whilst other studies have shown no significant differences in *Bifidobacterium* levels between elderly and younger adults *(6, 16)*. However, the low number of individuals included in most of the studies does not allow extrapolation to definite conclusions. In spite of these apparently contradictory results, the decline in bifidobacterial levels with age is still widely accepted and has been very commonly mentioned in recent studies *(5, 18)*. In order to clarify the bifidobacterial levels in the gut microbiota of elderly people, we decided to follow subjects of advanced age, close to their end stage of life, residing in nursing homes, to analyze fecal bifidobacterial numbers and to compare the concentrations in subjects who recently passed away to the concentrations in age- and sex- matched subjects in the same nursing homes. The major goal was to determine the bifidobacterial numbers in the elderly at the end stage of life, prior to death, and compare them with age- and sex-matched controls remaining alive for a longer time.

MATERIAL AND METHODS

Elderly patients (84 ± 1 years-old) in two nursing homes in Helsinki were screened and a cohort formed of volunteer subjects willing to participate were asked to provide fecal samples. Twenty-one subjects who died during the follow-up (within 4 months after sampling), were age- and sex-matched with control subjects in same nursing homes living for at least 6 months after the screening. This study formed the basis for a later intervention study and was accepted by the Ethics Committee of the City of Helsinki. Subjects and/or their guardians gave their written informed consent to participation in the study.

Faecal samples were frozen immediately after collection by the nursing staff and stored at –20°C until analysis. Faecal material was diluted 10 times in PBS buffer and homogenized in a Stomacher 400 (Seward Ltd, London, UK) at full speed for 2 min. Then, samples were analysed by using culture-dependent and culture independent methods. Bifidobacterial concentrations were assessed using the traditional plate count method and anaerobic incubation on Blood Liver agar (BL agar; Nissui Seiyaku, Tokyo). Isolated colonies with the appareance of bifidobacteria were microscopically confirmed and then counted. FISH analysis was performed by a previously described method *(11)*. The quantitative real-time PCR (qPCR) methodology described by Gueimonde et al. *(7)* for the quantification

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of intestinal bifidobacteria was also used to assess *Bifidobacterium* levels.

The zero values in the plate counting were changed to 10^4 (detection limit). The distributions were skewed and were $\log_{10}$ transformed before analysis. The paired samples t-test was used for matched pairs and the t-test for an independent sample was used for group comparisons of bifidobacterial levels. Pearson's χ^2 test was used to compare the occurrence of antibiotic use or different diseases between those who subsequently passed-away and those who stayed alive.

RESULTS AND DISCUSSION

No differences in antibiotic use, malnutrition problems or occurrence of common diseases among elderly people, including dementia, depression, Parkinson's disease, or coronary heart disease were found between the groups.

The traditional plate count method indicated fecal bifidobacteria levels at concentrations ranging from 10^4 to 10^9 cfu/g fecal contents in both subjects who died shortly after sampling and subjects who remained alive (mean ± sd; 6.18 ± 1.34 and 6.11 ± 1.50, respectively). The levels were within the range of those previously reported *(10, 15)*. The samples analyzed using FISH and qPCR produced results that, although slightly different, were not statistically different ($p > 0.05$). In general FISH produced slighly higher values than qPCR (Fig. 1). With both techniques the values obtained for the different subjects ranged from 10^7 to 10^{11} bacteria/g which is in agreement with previous observations of the elderly using real-time PCR *(2, 7)* and comparable to those found for healthy adults by using quantitative PCR and FISH *(13, 14)*. These values were significantly ($p < 0.05$) higher than the values observed in plate counting. In this regard, the freezing and thawing processes could have affected or damaged viable bifidobacteria killing them or inducing a dormant state rendering them unculturable by the plate count method *(4, 12)*. Another possible explanation is underestimation of *Bifidobacterium* populations in the elderly when using plate counts, since it has been shown that different culture media differ in the recovery of bifidobacteria *(1)*.

Fecal *Bifidobacterium* levels, determined by the molecular methods used (FISH and qPCR) (Fig. 1), were lower in the subjects who died shortly after sampling than in the corresponding controls although the differences were not statistically significant ($p = 0.430$ and 0.056 for FISH and qPCR, respectively).

The general conclusion is that fecal levels of bifidobacteria, as assessed by molecular methods, in the elderly at the end stage of life tend to decline when
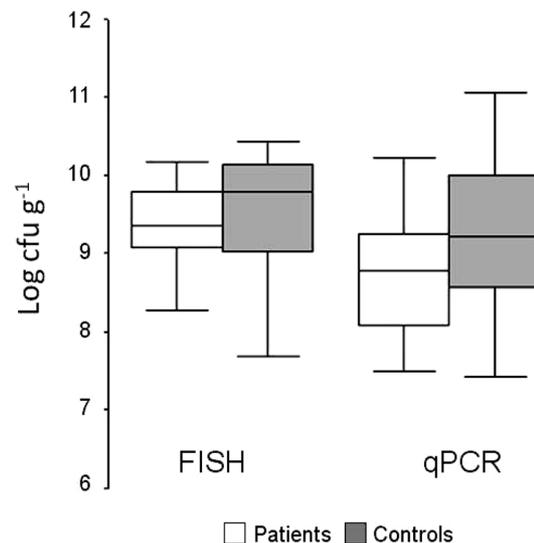

Fig.1 Bifidobacterial concentrations as determined by fluorescent in situ hybridization (FISH) and qPCR in patients (subjects who subsequently died) and controls (age- and sex-matched subjects staying alive). Black bars represent the median, the box represents the inter-quartile range and error bars the minimum and maximum values. No statistically significant diferences in bifidobacterial levels between patients and controls were found ($p = 0.430$ for FISH counts and $p = 0.056$ for qPCR counts)

compared to those found in age- and sex-matched controls remaining alive for a longer time. However, in general, the levels obtained were still high and in the range of those usually found in younger adults *(7, 13, 14)*. This suggests that the reduced bifidobacterial levels in elderly people observed by different authors using traditional culture methods do not appear to be confirmed by culture-independent techniques. Nevertheless, bifidobacteria among the elderly population may still have a significant role in gut health, in a manner similar to that in younger adults or infants. Understanding the metabolic activity of these components is thus needed to assess the potential of bifidobacterial supplementation for the elderly.

Taken together, a trend towards a reduction of bifidobacterial levels in elderly subjects at the very end stage of life appears to be present. Although no statistically significant differences were observed and the number of individuals included in this study was limited (21 per group), this trend may correlate with physiological decline at the end of life. Bifidobacterial levels should be carefully determined, with simultaneous assessment of the species composition of bifidobacteria microbiota during the late phase of life, in further studies.

Such characterization should provide detailed information on the activity of the bifidobacterial microbiota within the gastrointestinal tract and their health effects in old age. If aberrations in the bifidobacterial microbiota were to be found, new options for bifidobacterial supplementation could be developed to enhance the health and quality of life of the elderly.

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