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Evaluation of Faecal Zonulin and Calprotectin Levels in Healthy Children throughout the First Two Years of Life: A Prospective Observational Cohort Study

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Description

The study of biomarkers in faecal samples, such as zonulin and calprotectin, has provided critical insights into gut health and immune system activity during early childhood. Zonulin is a regulator of intestinal permeability, while calprotectin is a marker of intestinal inflammation. Monitoring these biomarkers during the first two years of life offers an opportunity to understand how gut health develops in healthy children, shedding light on normal physiological changes and potential early indicators of gastrointestinal disorders. This observational prospective cohort study aimed to evaluate the concentrations of faecal zonulin and calprotectin in healthy children throughout their first two years of life, providing a baseline for normal levels and patterns over time. The first two years of life are marked by rapid changes in a child's physiology, particularly in the gastrointestinal system. During this period, the gut undergoes significant development, influenced by factors such as diet transitions, microbial colonization, and immune system maturation. Zonulin, a protein linked to the regulation of tight junctions between intestinal epithelial cells, plays a key role in maintaining the gut barrier. Its dysregulation can lead to increased intestinal permeability, often referred to as "leaky gut," which has been associated with various conditions, including allergies, autoimmune disorders, and gastrointestinal diseases. On the other hand, calprotectin is a calcium- and zinc-binding protein released by activated neutrophils during inflammation [1]. Elevated levels of faecal calprotectin are indicative of intestinal inflammation and are used clinically to assess conditions such as inflammatory bowel disease. Understanding the normal ranges and fluctuations of these biomarkers in healthy children is essential for distinguishing pathological conditions from physiological variations during development.

This study recruited a cohort of healthy children and followed them prospectively from birth to two years of age. Stool samples were collected at regular intervals to measure the concentrations of faecal zonulin and calprotectin. The inclusion criteria ensured that all participants were healthy at the time of recruitment, with no known gastrointestinal diseases or significant medical conditions. Data on feeding practices (e.g., breastfeeding, formula feeding, and introduction of solids), antibiotic use, and other environmental factors were also collected, as these variables are known to influence gut health and biomarker levels. The results revealed dynamic patterns in the concentrations of both zonulin and calprotectin over the first two years of life. Zonulin levels were generally higher in the early months, reflecting the immaturity of the intestinal barrier in newborns [2]. This is consistent with the notion that the neonatal gut is more permeable to allow the transfer of maternal antibodies and other protective factors from breast milk. Over time, as the intestinal barrier matured, zonulin levels gradually decreased, reaching more stable levels by the end of the first year. This decline likely reflects the development of tighter junctions between epithelial cells, contributing to a more robust gut barrier. Factors such as breastfeeding appeared to influence zonulin levels, with breastfed infants showing slightly lower concentrations compared to formula-fed infants, possibly due to the protective and anti-inflammatory properties of breast milk.

Calprotectin levels, in contrast, showed a different pattern. Concentrations were highest during the neonatal period and early infancy, likely due to the physiological inflammatory response associated with gut colonization by microbes. The neonatal gut is sterile at birth, but it is rapidly colonized by bacteria, viruses, and fungi, a process that is critical for the development of a healthy microbiome and immune system. This colonization is accompanied by a transient inflammatory response, reflected in elevated calprotectin levels, as the microbiome stabilized and the immune system matured, calprotectin levels decreased, although occasional spikes were observed, potentially corresponding to periods of illness, dietary changes, or teething. By the second year of life, calprotectin levels in most children were within the lower range, indicative of a more stable and less inflammatory gut environment. The longitudinal design of this study allowed for the identification of individual variability in biomarker levels, highlighting the influence of external factors on gut health. Antibiotic use, for instance, was associated with temporary increases in both zonulin and calprotectin levels, likely due to the disruption of the microbiome and the associated inflammatory response. Similarly, the introduction of solid foods was a critical transition point, often accompanied by fluctuations in biomarker concentrations as the gut adapted to new dietary components. These findings underscore the importance of considering environmental and lifestyle factors when interpreting faecal biomarker data in young children [3].

The results of this study have important implications for clinical practice and research. Establishing reference ranges for faecal zonulin and calprotectin in healthy children provides a valuable baseline for identifying abnormal levels that may indicate underlying pathology. For example, persistently elevated zonulin levels beyond the first year of life could suggest impaired gut barrier function, warranting further investigation for conditions such as food allergies or celiac disease. Similarly, unusually high calprotectin levels may indicate ongoing inflammation, prompting evaluation for potential gastrointestinal disorders [4]. By distinguishing physiological variations from pathological changes, these biomarkers can serve as useful tools for early detection and monitoring of gut health issues in pediatric populations. Despite the valuable insights gained from this study, several limitations must be acknowledged. The cohort was composed of healthy children, and the findings may not be generalizable to populations with underlying medical conditions or different demographic characteristics. Additionally, while stool samples provide a non-invasive means of measuring gut health biomarkers, they may not fully capture the complexity of intestinal processes. Factors such as stool consistency and sampling methods could influence biomarker concentrations, introducing variability in the results. Future studies could benefit from larger, more diverse cohorts and the integration of additional biomarkers to provide a more comprehensive assessment of gut health [5].

This prospective observational cohort study provides a detailed characterization of faecal zonulin and calprotectin levels in healthy children during the first two years of life. The findings highlight the dynamic nature of these biomarkers, reflecting the rapid physiological changes in gut health and immune system development during early childhood. Zonulin levels gradually decrease as the intestinal barrier matures, while calprotectin levels decline following the initial inflammatory response to microbial colonization. These patterns establish a baseline for normal biomarker levels, aiding in the differentiation between physiological and pathological changes in

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pediatric gut health. By enhancing our understanding of these biomarkers, this study contributes to the broader effort to improve the early detection and management of gastrointestinal disorders in children.

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Conflict of Interest

None.

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