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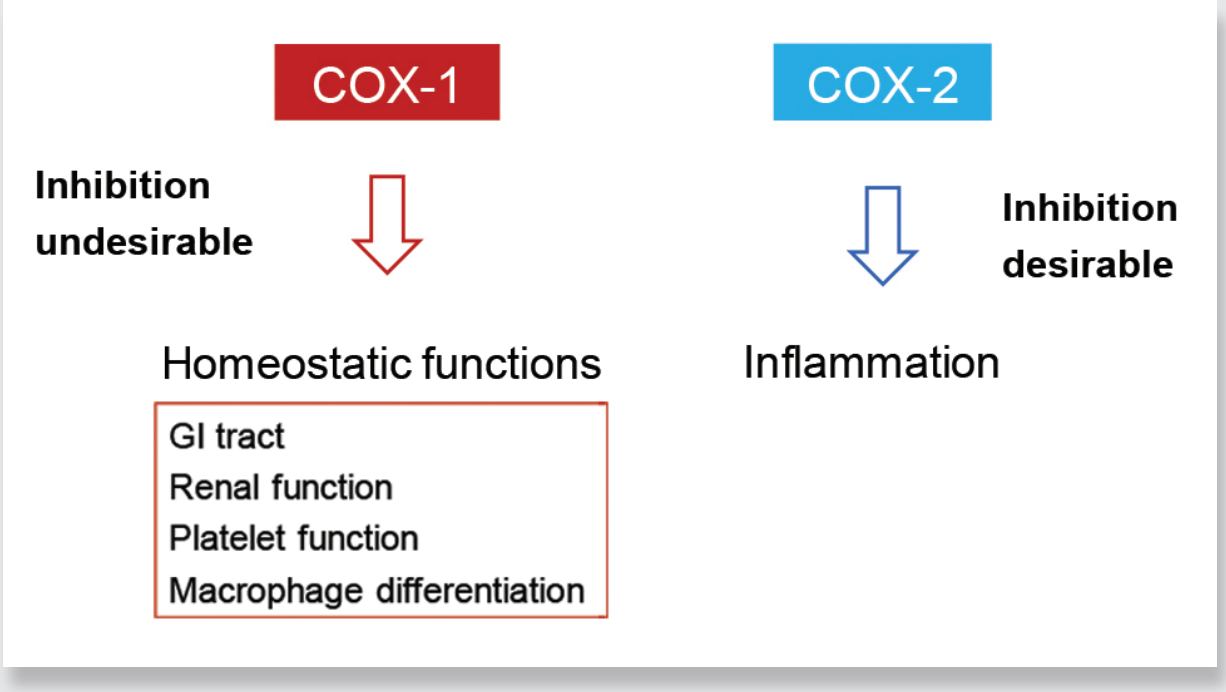

Isoforms of COX enzymes and their functions.

(See paper by Mermans et al)

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3. Jovanović S, Gajić I, Mandić B, Mandić J, Radivojević V. Oral lesions in patients with psychiatric disorders. Srp Arh Celok Lek 2010; 138:564–569. (Serbian)
4. Valença MM, Martins C, Andrade-Valença LPA. Trigeminal neuralgia associated with persistent primitive trigeminal artery. Migrâneas cefaléias (Brasil) 2008; 11:30–32.
5. Belenkaya RM. Structural variants of the brain base arteries. Vopr neirokhir 1974; 5:23–29. (Russian)

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Books:

7. Patten MB. Human embryology, 3rd edn. McGraw-Hill: New York, 1968.
8. Marinković S, Milisavljević M, Antunović V. Arterije mozga i kičmene moždine—Anatomske i kliničke karakteristike. Bit inženerenjering: Beograd, 2001. (Serbian)

Chapters:

9. Lie TA. Congenital malformations of the carotid and vertebral arterial systems, including the persistent anastomoses. In: Vinken PJ, Bruyn GW (eds) Handbook of clinical neurology, vol. 12. North Holland: Amsterdam, 1972; pp 289–339.

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10. Reed ML. *Si-SiO_2 interface trap anneal kinetics*, PhD thesis. Stanford University: Stanford, 1987.

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11. Apostolides PJ, Lawton MT, David CA, Spetzler RF. Clinical images: persistent primitive trigeminal artery with and without aneurysm. Barrow Quarterly 1997; 13(4). http://www.thebarrow.org/Education_And_Resources/Barrow_Quarterly/204843
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Editorial

DOES GUT MICROBIOME HOLD PROMISE OF LONGEVITY?

Austrian pediatrician, Ernst Moro (1874-1951), famous for his discovery of the infant Moro reflex, named after him, was also the first to publish that the breast-fed babies have a stronger bactericidal activity in their blood than bottle-fed ones, due to the presence of Lactobacillus acidophilus in human milk [1]. He isolated the bacterium from the stomach of naturally nourished children. This finding explained the better survival rate of these children during different, not only intestinal infections.

This early discovery evolved nowadays to a "secret" or "hidden organ", a new name applied for the gut microbiome harboring trillions of good bacteria, famous for its pleiotropic functions, influencing health and survival of human beings in the new era of different disrupters - plastic, antibiotics, food toxins and other challenges of modern life. The secret of longevity is studied in centenarians (mainly ladies) and consists of biased and amusing tips given by this small group, including: a sense of humor, quotidian chocolate consumption, dancing, daily bacon and eggs use, even ice-cream and whisky or vodka martini… Italian supercentenarian, lady Emma Morano (1899-2017) discovered a secret of her amazing life: 3 eggs daily, wine, chocolate and plenty of olive oil.

But what is modern medicine advising when it comes to healthy aging and longevity? Shortly: a lower calorie intake, staying lean, avoiding insulin resistance and type 2 diabetes, as well as the autoimmunity. And how to accomplish all these together? In the Victorian era, increasing population did not starve and longevity increased. Furthermore, the *per capita* numbers of significant scientific and technological innovations and also *per capita* numbers of scientific geniuses was dominant in the Victorian era, after which there was a decline. Phenotypic intelligence that results from a combination of genes and environmental factors like nutrition, hygiene, improved education and cognitive complexity increased during the Victorian times [2, 3].

Could we ameliorate our chances for longevity by manipulating another genome present in our gut? Namely, our gastrointestinal system is much more than just a digestion center. It is also home to a 70% of our immune system (GALT- gut associated lymphoid tissue) and our body's "second brain", due to a rich innervation system. Many vital funtions of the gut microbiome are well known, such as vitamin biosyntesis, bile acid degradation, maintenance of the intestinal mucosal barrier integrity, complex carbohydrate digestion, energy consumption and its allocation... Although neglected by the endocrinologists, gut microbiome represents an important endocrine organ that converts nutritional signals from the intestinal lumen into endocrine messages. Dopamine, norepinephrine, nitric oxide and the inhibitory transmiter GABA are molecules originating from the luminal microbes that influence our endogenous endocrine network ("Microbial endocrinology"). The gut bacteria could even deiodinate thyroid hormones, thus raising active serum fraction of these hormones [4, 5]. Leaky gut has been accused of initiation and promotion of autoimmune diseases. In an Invited article by Emma Hernandez Sanabria et al. you may discover how microbiome influences drug metabolism and activity.

Taking all together, we must put more attention to our cohabitants in the form of gut microbiome. Healthy gut means healthy individual and a perspective for a prolonged lifespan by fighting against important human pathology.

Acknowledgement: to Vladimir Vukovic, Institute for Biomedicine, Eurac Research, Affiliated Institute of the University of Lübeck, Bolzano, Italy, for thoughtful and useful comments.

References

1. Weirich A, Hoffmann GF. Ernst Moro (1874-1951)-A great pediatric career started at the rise of university-based pediatric research but was curtailed in the shadows of Nazi laws. Eur J Pediatr 2005; 164: 599-606.
2. Woodly MA, Nijenhuis J, Murphy R. Were the Victorians cleverer than us? The decline in general intelligence estimated from a meta-analysis of the slowing of simple reaction time. Intelligence 2013, http://dx.doi.org/10.1016/j.intell.2013.04.006
3. Duntas LH. The Thyroid under threat in the world of plastics. Facta Universitatis Series Medicine and Biology 2017; 19(2): 47-50.
4. Lerner Aaron, Jeremias Patricia, Matthias T. Gut-thyroid axis and celiac disease. Endocrine Connections 2017, 6: R52-R58.
5. Motta BM, Grander C, Gogele M, Foco L, Vukovic V, Melotti R et al. Microbiota, type 2 diabetes and non-alcocholic fatty liver disease: protocol of an observational study. J Transl Med 2019 17:408, https://doi.org/10.1186/s12967-019-02130-z

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Invited Review Article

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AS THERAPEUTIC ALLIES OF THE GUT MICROBIOME ON CHRONIC INFLAMMATION

Fabian Mermans, Evelien Heiremans, Maud Van Belleghem, Axelle Meersschaut, Emma Hernandez-Sanabria

Center for Microbial Ecology and Technology (CMET), Ghent University, Ghent, Belgium

Abstract. *Our gut harbours around 10^{14} bacteria of more than 1000 species, accounting for approximately 2 kg of biomass. The gut microbiome plays several vital functions in processes such as the development of the immune system, food digestion and protection against pathogens. For these functions to be beneficial for both host and microbiome, interactions are tightly regulated. Gut and immune cells continuously interact to distinguish among commensal microbiota, harmless foodstuff, and pathogens. A fine balance between inflammatory and anti-inflammatory state is fundamental to protect intestinal homeostasis. Nonsteroidal anti-inflammatories (NSAIDs) are a class of drugs used for management of pain and inflammation. These compounds have heterologous structures but similar therapeutic activities. The target of all NSAIDs are the isoforms of cyclooxygenase enzymes (COX): the primarily constitutive form COX-1, and the inducible from COX-2. Both isoforms catalyse the conversion of arachidonic acid to PGH2, the immediate substrate for specific prostaglandin and thromboxane synthesis. The gut microbiota plays a role in drug metabolism, resulting in altered bioavailability of these compounds. Additionally, complex host-microbiome interactions lead to modified xenobiotic metabolism and altered expression of genes involved in drug metabolism. These effects can be at gut tissue-level, or distant, including in the liver. Besides the gut microbiome influencing drug metabolism, drugs also impact the microbial communities in the gut. As different drugs exert selective pressures on the gut microbiome, understanding this bidirectional relationship is crucial for developing effective therapies for managing chronic inflammation.*

Key words: *gut microbiome, non-steroidal anti-inflammatories, inflammation, dysbiosis.*

Introduction

The gut microbiota includes more than 100 trillion bacterial cells [1], which can be divided into six dominant phyla: Firmicutes, Bacteroidetes and Actinobacteria, and the less abundant Proteobacteria, Fusobacteria and Verrucomicrobia [2]. The human microbiome is not only composed of collective genomes from bacteria, but also from those of archaea, viruses, and eukaryotes colonizing the gut [3]. The composition of the microbiota will depend on environmental factors, diet, age and host genetics [4]. Inter- and intra-individual differences are significantly prevalent, although several species such as *Faecalibacterium prausnitzii*, *Roseburia intestinalis* and *Bacteriodes uniformis* have been consistently identified in large scale screenings. The gut microbiome transforms indigestible substrates from food, such as xyloglucans, into short chain fatty acids, which can be used as energy source for the colonocytes [5]. These metabolic activities may directly or indirectly impact health and disease in the host. The commensal microbiota can train the immune system to induce regulatory T cells, which then prompt tolerance to these bacteria [6]. In this way, commensal-induced immunity contributes to maintain mucosal tolerance and protect the host from disease [7]. Such exchanges are tightly regulated: gut and immune cells continuously interact to distinguish between commensal microbiota, harmless foodstuff, and pathogens. A fine balance between inflammatory and anti-inflammatory status is needed to preserve intestinal homeostasis [8].

Interest in developing colon-targeted drug delivery systems has increased in recent years [9]. These formulations are intended for gastrointestinal-related diseases, such as colorectal cancer (CRC) and inflammatory bowel disease (IBD). Despite the numerous research in this field, there are still questions that need to be answered, as differences in microbiome composition may ultimately impact drug metabolism between subjects [10]. For example, which characteristics of the microbiome contribute to the pharmacokinetic differences between individuals? Does the region in the colon reflect a specific colonization pattern, which will later trigger metabolic differences? Do long-term alterations in the gut microbiome generate metabolic dysbiosis? If so, to what extent? Gaining insight into the gut metabolic potency is essential, because characterization of the players involved in drug disposition in the gut will contribute to ensure optimal drug efficacy and safety profile.

Existing colon-specific drug delivery systems hold some limitations. Besides the microbial influence on

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drug bioavailability and pharmacokinetics, low solubility in presence of bile acids and the small aqueous volume in the colon may hinder drug efficacy [11]. Moreover, understanding drug transporter activity is fundamental to improve drug passive permeability. Current models to predict drug efficacy consider plasma concentrations to describe the pharmacokinetics and pharmacodynamics. However, drugs designed for targeting the gut undergo an extensive first pass metabolic clearance. This implies that the relation between plasma concentrations and efficacy has a low predictive value. For this reason, investigating microbiome-drug-host interactions towards improving ADME and safety responses of intestinal targets is fundamental [12].

Xenobiotic Metabolism of the Gut Microbiome

Chemotherapeutic outcomes are mainly linked to human genetic polymorphisms [13], but the impact of the human gut microbiome has been overlooked, as it has been even called the "forgotten organ". Although microbiome research has flourished in recent years, the association between the microbiota and xenobiotics metabolism remains underexplored. The host cytochrome CYP2C9 predominantly eliminates lipophilic drugs through the liver, whereas orally administered drugs encounter the gut microbiota before reaching host tissues [10]. This first-pass metabolism by the colonic microbiota must be considered, as the metabolites generated from this process can impact drug activity, resulting in toxicity for the host and further inflammation, or altering gut microbiota and producing dysbiosis [14].

The anaerobic environment of the human gut prevents the microbial use of oxygen as terminal electron acceptor for oxidation [10, 14]. Thus, anaerobic respiration and increase in microbial growth are facilitated by the two main metabolic transformations: reduction provides alternative electron acceptors, while hydrolysis ensures substrates that can be used by microorganisms [15]. Since these two reaction types are commonly observed, core microbial species or core gene families performing such functions are hypothesized to participate [16].

Links between gut bacteria and drug metabolism are via direct and indirect mechanisms. Direct mechanisms involve the transformation of the drug into metabolites with a different effect. Conversely, indirect mechanisms are those influencing drug transport or metabolism [14]. Knowledge of these metabolic pathways is fundamental to predict whether drug metabolism and disposal will be impacted.

Additionally, biliary excretion is essential for drug recirculation and ultimate metabolism. In this respect, β-glucuronidases can play a key role, because they are important for host drug detoxification [17]. Glucuronic acid is coupled to several substrates in the liver, thereby increasing molecular weight and solubility. Consequently, elimination through urine or faeces is enhanced, and gut bacteria can reactivate these products, generating higher toxicity. Likewise, non-steroidal anti-inflammatory drugs (NSAIDs) have side-effects [18]. Microbial β-glucuronidases can separate the aglycone from the glucuronide, so the aglycone is absorbed into the enterocytes and transformed into reactive metabolites, harming the mitochondria and the endoplasmic reticulum. In this way, not only the host detoxification pathway is diminished, but also inflammation is evoked as a result of decreased mucosal integrity [19]. The impact of the gut microbiota on hepatic drug-processing genes is enzyme-specific and age and sex-dependent, with patterns varying throughout life span and developmental periods, and xenobiotic pathways significantly downregulated in male mice at 90 days of age [20].

Moreover, probiotic interventions have confirmed that microbiome manipulations not involving antibiotics can influence the expression of hepatic drug–processing genes during adulthood [21]. Poorly absorbed antibiotics such as vancomycin and rifaximin, may indirectly modulate hepatic CYP gene expression, as they bypass hepatic metabolism and impact gut bacteria [22]. Understanding the outcomes of indirect host–microbiome-drug interactions occurring because of drug use, consumption of dietary supplements, or environmental toxins should be considered in drug development, safety pharmacology, and pharmacokinetic profiling [20].

The Gut Microbiome and Xenobiotic Metabolism during Chronic Inflammation

Although research efforts are ongoing to more precisely define a healthy gut microbiome [23], one of the most frequently reported findings across an array of disorders is a narrowing of gut microbiome diversity often accompanied by more specific but less consistent compositional alterations at various taxonomic levels. Dysbiosis refers to the state where the composition of the microbiome is disrupted [24, 25], providing continuous immunological stimulation and leading to anomalies in the immune response. The polarized induction of immune cells and the subsequent increased amounts of pro-inflammatory T cells may fuel the development and severity of the disease. Excessive inflammation results in loss of epithelial integrity, which in turn leads to further bacterial translocation and thus further induction of inflammation [8, 24]. This condition is observed in a number of gastrointestinal diseases such as inflammatory bowel disease (IBD) and colorectal cancer (CRC) [26], central nervous system disorders such as depression and schizophrenia [27], obesity [28], diabetes mellitus [29], cardiovascular disorders [30], rheumatoid arthritis [31] and multiple sclerosis [24], and asthma and atopy in children [32]. Research found that *Faecalibacterium prausnitzii* is reduced in patients with ulcerative colitis, another chronic inflammatory condition of the colon [33]. This bacterium has been reported to promote the accumulation of regulatory T cells (T_{reg}), which contribute to anti-inflammatory responses [34]. On the

contrary, Enterobacteriaceae, Bacteroides, *Clostridium ramosum* and *Akkermansia muciniphila* are increasingly present in patients with IBD. These organisms have also been linked with increased inflammation [35]. Although the importance of the gut microbiome in chronic inflammatory conditions has been reported [36], it is still unclear whether dysbiosis is a consequence or a cause of associated diseases [8, 24].

Gut microbiota are known to play a role in the metabolism of drugs, hereby having an effect on the host health . Many of the therapeutic interventions for chronic disorders are subject to biotransformation by the gut microbiome, and the functional implications of changes in gut bacterial communities for xenobiotic metabolism are yet to be described. The gut microbiome can affect drug therapy via direct or indirect mechanisms. The direct mechanisms compromise metabolization of the drugs by the microbiota, resulting in altered bioavailability of these compounds. Indirect mechanisms include complex host-microbiome interactions, resulting in a modified xenobiotic metabolism of the host [10]. In the latter case, one of the routes is that microbiota affect host gene expression involved in drug metabolism. These effects can be either local, meaning in the gut tissue [37], or distant, including in the liver [38].

Understanding how alterations in gut microbiota profiles influence host response to chemotherapeutic drugs may have important clinical implications. Stratifying patients based on their gut microbiome composition may assist in identifying responders and non-responders to immunotherapy [39] for the treatment of epithelial tumours and melanomas [20, 40]. Other mechanisms modulated by the gut microbiome include the translocation and immunomodulation following interventions with cyclophosphamide, doxorubicin, and anti–CLTA-4 therapies [41].

Dynamics of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) towards the Gut Microbiome

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a class of drugs used for inflammation management. The compounds in this group are heterologous in structure but show similar therapeutic activity [42]. The target of all NSAIDs are cyclooxygenase enzymes (COX). There are two isoforms of COX, the primarily constitutive form COX-1, and the inducible from COX-2 [42]. COX-1 and COX-2 both catalyse the conversion of arachidonic acid to PGH_2 [43]. PGH_2 is the immediate substrate for some cell specific prostaglandin and thromboxane synthases [42].

COX-1 is expressed in different tissues such as the kidneys, lungs, stomach, ileum and colon [44]. It is considered to be a “housekeeping” enzyme, playing an important role in the production of prostaglandins that serve homeostatic functions [45]. These prostaglandins, among others, maintain the integrity of the gastric mucosa, mediate normal platelet function and regulate renal blood flow [46]. COX-2 on the other hand, is normally not expressed in tissues with the exception of the macula densa of the brain and kidney, the placenta, the vessel walls and the heart, where it is believed to play a role in homeostatic functions [47]. This isoform of the enzyme is upregulated in response to tissue damage or the presence of pro-inflammatory cytokines [43]. In turn, COX-2 is responsible for the generation of pro-inflammatory prostaglandins, thus contributing to inflammation [45]. One of these prostaglandins is PGE_2, which is implicated in carcinogenesis. COX-2 expression is found to be increased in colorectal cancer causing proliferation, enhanced angiogenesis and suppression [48] of apoptosis (Pugh & Thomas, 1994; Surh et al., 2001).Thus, COX-2 is the main target of chronic inflammation therapy using non-steroidal anti-inflammatory drugs (Fig. 1).

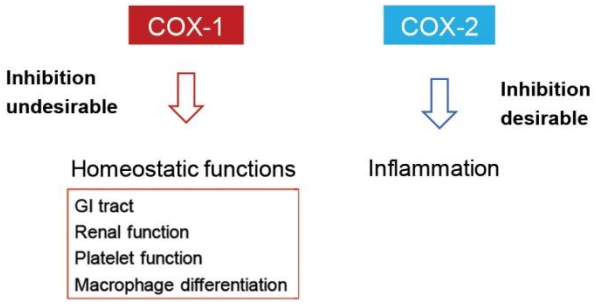

Fig. 1 Isoforms of COX enzymes and their functions

Orally administered drugs are subject to the so called “first-pass metabolism”, where the gut microbiome plays a crucial part. Prior to reaching the target tissue, microbiota and liver metabolize the drugs, altering their bioactivity [10]. The bacterial metabolism results in three scenarios, namely activation, inactivation, and increased toxicity of the drug. In the first situation, the pharmaceutical compound is activated into its therapeutic window, resulting in increased drug efficacy. An example is the described transformation of sulindac to its bioactive sulphide metabolite. In the case of inactivation, the availability of the active drug is lowered resulting in decrease or loss of therapeutic effect. For instance, microbial arylamine N-acetyltransferases inactivate the bioactive component of the anti-inflammatory drug sulfasalazine [14], while the NSAID diclofenac can exert toxic effects after being glucuronidated in the liver by the host and then being exposed to microbial β-glucoronidases in the gut [10, 18]. In these scenarios, activity of the gut microbiome results in increased toxicity and adverse effects for the host. In addition, simultaneous use of probiotics significantly increased the microbiota-mediated enzymatic degradation of the antipyretic and analgesic paracetamol [20, 39]. Similarly, short-term administration of a probiotic cocktail of *L. acidophilus*, *B. lactis* and *Streptococcus salivarius* to rats significantly increased azoreductase activity in *ex vivo* incubation of sulfasalazine with colon contents, ultimately impacting the metabolism of this drug [49].

Besides the gut microbiome influencing drug metabolism, drugs also impact the microbial communities in the gut. As discussed earlier, a shift in gut microbiome, and more specifically dysbiosis, is linked to disease. Therefore, understanding the effects of drugs on the gut microbiota is crucial for providing suitable therapies [14]. Obvious examples include the use of antibiotics, resulting in altered gut microbiome [50]. NSAIDs have been reported to impact the gut microbiome and changes in composition have been observed [51] depending on the NSAID administered. Subjects exposed to aspirin showed greater abundance in *Prevotella* spp., *Bacteroides* spp., *Barnesiella* sp. and Ruminococcaceae. Celecoxib and ibuprofen users showed enrichment in Enterobacteriaceae and Acidaminococcaceae. Ibuprofen users were also enriched in Propionibacteriaceae, Pseudomonadaceae, Puniceicoccaceae and Rikenellaceae [52]. In contrast, a different study observed no effects on the microbiome composition after administration of celecoxib [53]. Sulfasalazine induced the expression of thioredoxins and nitrate reductases, while nizatidine, subject to bacterially mediated N-oxide bond cleavage, up-regulated the expression of drug enzymes and transporters acting on nitrogen bonds [54]. This finding supports the earlier hypothesis that drugs may shift the microbiota to favour the abundance of taxa involved in its metabolism. Furthermore, this altered metabolic capacity of the microbiota could consequently affect not only the pharmacokinetics of subsequent doses of the drug itself (a phenomenon referred to as autoinduction) but also the pharmacokinetics of co-administered medication may be act as substrates of the same metabolic pathway or transporter. Production of diet-derived by-products such as like *p*-cresol (from protein digestion) can affect drug metabolism. Research showed that acetaminophen, an analgesic and antipyretic drug, underwent less sulfonation due to competitive O-sulfonation of *p*-cresol [55].

COX-1 plays a role in guaranteeing the mucosal integrity of the gastrointestinal tract and thus, non-specific NSAIDs blocking both isoforms of COX have been linked with intestinal damage [19]. These environmental changes place a selective pressure on the microbiome resulting in fluctuations in microbial composition [56]. Blocking intestinal bacterial enzymatic functions has been proposed to amend intestinal homeostasis, while enhancing efficacy and decreasing toxicity of IBD therapies, as realised for cancer therapies [4, 17]. Precision editing of the gut microbiota by tungstate ameliorates experimental gut inflammation through preventing the dysbiotic expansion of Enterobacteriaceae [57]. This is merely an example of the complex host-microbiome-drug interactions and it is likely that different drugs exert selective pressures on the gut microbiome through different mechanisms [14]. The use of designer lactic acid bacteria as factories for the production of antimicrobial and anti-inflammatory biomolecules may also have potential for the future treatment of infectious diseases, cancer, and metabolic diseases [54, 58].

Conclusion

It is clear that the gut microbiome play an enormous role in patient health, but also in pharmacokinetic and pharmacodynamic drug response. Before official approval, government agencies such as the FDA run three phases of clinical trials, where pharmacokinetics, pharmacodynamics, and safety of a drug are determined. However, gut bacteria is not currently considered within these evaluations, although the microbiome is the first checkpoint following drug intake. *In vitro* studies have shown that some NSAIDs can be metabolized by bacteria, potentially jeopardizing its anti-inflammatory effects. More than 60 interactions between drugs and the microbiome have already been documented. Unfortunately, most of the underlying molecular and genetic mechanisms remain unclear. The PharmacoMicrobiomics online database was launched in 2011 to assemble the literature about those interactions [59]. Future efforts may focus on combining available information about the drug-bug interactions on biochemical pathways with existing genomic, pharmacogenomic and human microbiome sequence databases [52].

Personalized medicine has achieved excellent results in some areas of medicine, such as oncology [4]. Yet, genetic factors can influence up to 50% of therapy responses [13]. It is at this crossroads that recent discoveries on the roles of gut microbiota have become paramount [60]. Stratification of patients based on their gut microbiome may improve treatment accuracy [17] and cost-effectiveness [61]. A combined strategy to restore eubiosis may require synchronised targeting of dominant pathobionts and replacing missing beneficial species or their functions by manipulating the bacterial microbiota with dietary strategies. Thus, homeostatic immune responses, and mucosal barrier function would be reconditioned. This integrated approach may result safer than the current lifelong treatments with immunosuppressive drugs, once remission has been accomplished by traditional therapies.

Understanding how our "second genome" [4] is involved in therapeutic responses could pave the way for approaches using the intestinal microbiome as the target for modulating drug efficacy and enabling tailored treatments. Revealing the specific mechanisms driving defective bacteria–host interactions will enable precision editing of gut microbiota functionality and composition for ameliorating chronic inflammatory diseases.

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References

1. Valdes AM, Walter J, Segal E, Spector TD. Role of the gut microbiota in nutrition and health. BMJ 2018; 361:k2179.
2. Zhao L, Zhang X, Zuo T, Yu J. The composition of colonic commensal bacteria according to anatomical localization in colorectal cancer. Engineering 2017; 3:90-97.
3. Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S. The evolution of the host microbiome as an ecosystem on a leash. Nature 2017; 548:43-51.
4. Zmora N, Zeevi D, Korem T, Segal E, Elinav E. Taking it personally: personalized utilization of the human microbiome in health and disease. Cell Host Microbe 2016; 19:12-20.
5. De Vuyst L, Leroy F. Cross-feeding during human colon fermentation. In: González-Ortiz G, Bedford MR, Bach Knudsen KE, Courtin CM, Classen HL, editors. The value of fibre: Engaging the second brain for animal nutrition. Wageningen Academic Publishers; 2019. pp 565-569.
6. Kato LM, Kawamoto S, Maruya M, Fagarasan S. The role of the adaptive immune system in regulation of gut microbiota. Immunol Rev 2014; 260:67-75.
7. Lathrop SK, Bloom SM, Rao SM, Nutsch K, Lio C-W, Santacruz N, Peterson DA, Stappenbeck TS, Hsieh C-S. Peripheral education of the immune system by colonic commensal microbiota. Nature 2011; 478:250.
8. Shen S, Wong CH. Bugging inflammation: role of the gut microbiota. Clin Transl immunology 2016; 5:e72.
9. Amidon S, Brown JE, Dave VS. Colon-targeted oral drug delivery systems: design trends and approaches. Aaps PharmSciTech 2015; 16:731-741.
10. Spanogiannopoulos P, Bess EN, Carmody RN, Turnbaugh PJ. The microbial pharmacists within us: a metagenomic view of xenobiotic metabolism. Nat Rev Microbiol 2016; 14:273-87.
11. McConnell EL, Fadda HM, Basit AW. Gut instincts: explorations in intestinal physiology and drug delivery. Int J Pharm 2008; 364:213-226.
12. Hernandez-Sanabria E, Heiremans E, Arroyo MC, Props R, Leclercq L, Snoeys J, et al. Short term supplementation of celecoxib shifted butyrate production on a simulated model of the gut microbial ecosystem and ameliorated in vitro inflammation. bioRxiv 2019; 679050.
13. Tannock IF, Hickman JA. Limits to personalized cancer medicine. N Engl J Med 2016; 375:1289-1294.
14. Wilson ID, Nicholson JK. Gut microbiome interactions with drug metabolism, efficacy, and toxicity. Transl Res 2017; 179:204-222.
15. Arkhipova OV, Akumenko VK. Unsaturated organic acids as terminal electron acceptors for reductase chains of anaerobic bacteria. Microbiology 2005; 74:629-639. [In Russian]
16. Haiser HJ, Seim KL, Balskus EP, Turnbaugh PJ. Mechanistic insight into digoxin inactivation by Eggerthella lenta augments our understanding of its pharmacokinetics. Gut Microbes 2014; 5:233-238.
17. Kent DM, Steyerberg E, van Klaveren D. Personalized evidence based medicine: predictive approaches to heterogeneous treatment effects. BMJ 2018; 363:k4245.
18. Higuchi K, Umegaki E, Watanabe T, Yoda Y, Morita E, Murano M, et al. Present status and strategy of NSAIDs-induced small bowel injury. J Gastroenterol 2009; 44:879-888.
19. Boelsterli UA, Redinbo MR, Saitta KS. Multiple NSAID-induced hits injure the small intestine: underlying mechanisms and novel strategies. Toxicol Sci 2012; 131:654-667.
20. Clarke G, Sandhu KV, Griffin BT, Dinan TG, Cryan JF, Hyland NP. Gut reactions: breaking down xenobiotic–microbiome interactions. Pharmacol Rev 2019; 71:198-224.
21. Selwyn FP, Cheng SL, Bammler TK, Prasad B, Vrana M, Klaassen C, et al. Developmental regulation of drug-processing genes in livers of germ-free mice. Toxicol Sci 2015; 147:84-103.
22. Gonzalez FJ, Jiang C, Patterson AD. An intestinal microbiota–farnesoid X receptor axis modulates metabolic disease. Gastroenterology 2016; 151:845-859.
23. Zhernakova A, Kurilshikov A, Bonder MJ, Tigchelaar EF, Schirmer M, Vatanen T, et al. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. Science 2016; 352:565-569.
24. Forbes JD, Van Domselaar G, Bernstein CN. The gut microbiota in immune-mediated inflammatory diseases. Front Microbiol 2016; 7:1081.
25. Slingerland AE, Schwabkey Z, Wiesnoski DH, Jenq RR. Clinical evidence for the microbiome in inflammatory diseases. Front Immunol 2017; 8:400.
26. Sobhani I, Tap J, Roudot-Thoraval F, Roperch JP, Letulle S, Langella P, et al. Microbial dysbiosis in colorectal cancer (CRC) patients. PloS One 2011; 6:e16393.
27. Rogers G, Keating D, Young R, Wong M, Licinio J, Wesselingh S. From gut dysbiosis to altered brain function and mental illness: mechanisms and pathways. Mol Psychiatry 2016; 21:738.
28. Cani PD, Delzenne NM. Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota. Curr Opin Pharmacol 2009; 9:737-743.
29. Li X, Watanabe K, Kimura I. Gut microbiota dysbiosis drives and implies novel therapeutic strategies for diabetes mellitus and related metabolic diseases. Front Immunol 2017; 8:1882.
30. Yang T, Santisteban MM, Rodriguez V, Li E, Ahmari N, Carvajal JM, et al. Gut dysbiosis is linked to hypertension. Hypertension 2015; 65:1331-1340.
31. Maeda Y, Takeda K. Role of gut microbiota in rheumatoid arthritis. J Clin Med 2017; 6:60.
32. Lynch SV, Pedersen O. The human intestinal microbiome in health and disease. N Eng J Med 2016; 375:2369-2379.
33. Sokol H, Seksik P, Furet J, Firmesse O, Nion-Larmurier I, Beaugerie L, et al. Low counts of Faecalibacterium prausnitzii in colitis microbiota. Inflamm Bowel Dis 2009; 15:1183-1189.
34. Qiu X, Zhang M, Yang X, Hong N, Yu C. Faecalibacterium prausnitzii upregulates regulatory T cells and anti-inflammatory cytokines in treating TNBS-induced colitis. J Crohns Colitis 2013; 7:e558-e568.
35. Gkouskou K, Deligianni C, Tsatsanis C, Eliopoulos AG. The gut microbiota in mouse models of inflammatory bowel disease. Front Cell Infect Microbiol 2014; 4:28.
36. Clemente JC, Manasson J, Scher JU. The role of the gut microbiome in systemic inflammatory disease. BMJ 2018; 360:j5145.
37. Schroeder BO, Bäckhed F. Signals from the gut microbiota to distant organs in physiology and disease. Nat Med 2016; 22:1079.
38. Swanson HI. Drug metabolism by the host and gut microbiota: a partnership or rivalry? Drug Metab Dispos 2015; 43:1499-1504.
39. Mullard A. Oncologists tap the microbiome in bid to improve immunotherapy outcomes. Nat Rev Drug Discov 2018; 17:153-155.
40. Viennois E, Gewirtz AT, Chassaing B. Chronic inflammatory diseases: Are we ready for microbiota-based dietary intervention? Cell Mol Gastroenterol Hepatol 2019; 8:61-71.
41. Alexander JL, Wilson ID, Teare J, Marchesi JR, Nicholson JK, Kinross JM. Gut microbiota modulation of chemotherapy efficacy and toxicity. Nat Rev Gastroenterol Hepatol 2017; 14:356-365.
42. Carbone C, Musumeci T, Pignatello R. Non-steroidal anti-inflammatory drugs. In: Pignatello R, editor. Drug-Biomembrane Interaction Studies.The Application of Colorimetric Techniques. Cambridge: Woodhead Publishing; 2013. p 281-303,
43. Funk CD, FitzGerald GA. COX-2 inhibitors and cardiovascular risk. J Cardiovasc Pharmacol 2007; 50:470-479.
44. Wang D, DuBois RN. The role of COX-2 in intestinal inflammation and colorectal cancer. Oncogene 2010l 29:781-788.
45. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, A. Van De Putte LB, Lipsky PE. Cyclooxygenase in biology and disease. FASEB J 1998; 12:1063-1073.
46. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol 2011; 31:986-1000.
47. Mullins MN, Lana SE, Dernell WS, Ogilvie GK, Withrow SJ, Ehrhart E. Cyclooxygenase-2 expression in canine appendicular osteosarcomas. J Vet Intern Med 2004; 18:859-865.
48. Kang M, Martin A. Microbiome and colorectal cancer: Unraveling host-microbiota interactions in colitis-associated colorectal cancer development. Semin Immunol 2017;32:3-13.

49. Lee HJ, Zhang H, Orlovich DA, Fawcett JP. The influence of probiotic treatment on sulfasalazine metabolism in rat. Xenobiotica 2012; 42:791-797.
50. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci U S A. 2011;108 Suppl 1:4554-61.
51. Mäkivuokko H, Tiihonen K, Tynkkynen S, Paulin L, Rautonen N. The effect of age and non-steroidal anti-inflammatory drugs on human intestinal microbiota composition. Brit J Nutr 2010; 103:227-234.
52. Rogers MA, Aronoff DM. The influence of non-steroidal anti-inflammatory drugs on the gut microbiome. Clin Microbiol Infect 2016; 22:178. e1-178. e9.
53. Bokulich NA, Battaglia T, Aleman JO, Walker J, Blaser MJ, Holt PR. Celecoxib does not alter intestinal microbiome in a longitudinal diet-controlled study. Clin Microbiol Infect 2016; 22:464-465.
54. Walsh J, Griffin BT, Clarke G, Hyland NP. Drug–gut microbiota interactions: implications for neuropharmacology. Br J Pharmacol 2018; 175:4415-4429.
55. Clayton TA, Baker D, Lindon JC, Everett JR, Nicholson JK. Pharmacometabonomic identification of a significant host-microbiome metabolic interaction affecting human drug metabolism. Proc Natl Acad Sci U S A 2009; 106:14728-14733.
56. Tjalsma H, Boleij A, Marchesi JR, Dutilh BE. A bacterial driver–passenger model for colorectal cancer: beyond the usual suspects. Nat Rev Microbiol 2012; 10:575-582.
57. Zhu W, Winter MG, Byndloss MX, Spiga L, Duerkop BA, Hughes ER, et al. Precision editing of the gut microbiota ameliorates colitis. Nature 2018; 553:208-211.
58. Piard JC, Briandet R. Lactic acid bacteria biofilms: from their formation to their health and biotechnological potential. In: Mozzi F, Raya RR, Vignolo GM. Biotechnology of Lactic Acid Bacteria: Novel Applications: 2nd ed. West Sussex, UK: John Wiley & Sons, Ltd; 2015. p. 341-361.
59. ElRakaiby M, Dutilh BE, Rizkallah MR, Boleij A, Cole JN, Aziz RK. Pharmacomicrobiomics: the impact of human microbiome variations on systems pharmacology and personalized therapeutics. OMICS 2014; 18:402-414.
60. Routy B, Le Chatelier E, Derosa L, Duong CP, Alou MT, Daillère R, et al. Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. Science 2018; 359:91-97.
61. Goossens N, Singal AG, King LY, Andersson KL, Fuchs BC, Besa C, et al. Cost-Effectiveness of Risk Score–Stratified Hepatocellular Carcinoma Screening in Patients with Cirrhosis. Clin Transl Gastroenterol 2017; 8:e101.

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Review Article

PAI -1 INHIBITOR AS BIOMARKER OF CARDIORENAL DAMAGE

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Abstract. *Plasminogen activator inhibitor 1 (PAI-1), which belongs to the family of serine protease inhibitors is the primary regulator of plasminogen activity. PAI-1 is synthesized as a single-chain glycopeptide and is deposited in the platelets in a latent form, from which it is released upon their activation. It is spontaneously converted into stable molecules, unless it reacts with proteins from the plasma. As a powerful inhibitor of fibrinolysis, it participates in the pathogenesis of endothelial damage, processes of accelerated atherosclerosis and thromboembolism. All the diseases of the cardiovascular system which are dominated by the processes of fibrosis and thrombosis lead to an increase in PAI-1 in the circulation. In the emergence and development of atherosclerosis, it plays a role not only in the formation of intraluminal thrombus but also in neointimal proliferation. PAI-1 is not normally present in kidney tissue, but its concentration increases significantly in its acute and chronic kidney disease, thanks to the synthesis by the intrarenally localized inflammatory cells. In addition to genetic predisposition, the factors that directly influence the production of PAI-1 are the following: glycemia, insulin and various neurohumoral factors. It is not normally present in the kidney tissue, but its intrarenal concentration increases significantly in acute and chronic kidney diseases. Numerous studies have confirmed the significant role of PAI-1 in the development of diabetes complications. During the last decade there has been a growth of interest in the introduction of non-invasive methods or biomarkers that would assess the degree of fibrosis in the kidney. Many studies have confirmed association between kidney and heart disease. It is not only that these diseases share common risk factors, but many other mechanisms have been suggested. Plasminogen activator inhibitor -1 plays a role in the pathogenesis of endothelial damage, processes of fibrosis and thrombosis, development of diabetes complications and acute and chronic kidney diseases.*

Key words: *cardio-renal syndrome, biomarkers, plasminogen activator inhibitor-1*

Introduction

Brakman was the first to describe the existence of an inhibitor of tissue plasminogen activator in 1966 and only after 20 years of activator inhibitor-1 tissue plasminogen activator (PAI-1) was isolated from cultures of endothelial cells by which it is named an endothelial type plasminogen inhibitor of tissue. It belongs to the superfamily of serine protease inhibitors - serpin. It has four different structural forms with the conversion of certain regulatory mechanisms of fibrinolytic system. Its structure and site of synthesis (the endothelium, megakaryocytes, endometrium, macrophages, mesothelial cells, adipose tissue) were later determined. The gene responsible for the synthesis of PAI-1 was localized to chromosome 7. Once synthesized PAI-1 accumulates in platelets, it can be found in subendothelial matrix and in the bloodstream. Fibrinolytic system includes a wide spectrum of proteolytic enzymes and functions in the process of hemostasis, tissue remodeling, tumor invasion and angiogenesis. The main enzyme in the system is plasminogen activator, plasmin, which is responsible for degradation of soluble fibrin degradation products. Activation of plasminogen to plasmin is carried out by two types of activators: urokinase type plasminogen activator (uPA) and tissue-type activator plasminogen (tPA) and fibrin. Clearance and its accumulation are regulated by specific inhibitors of plasminogen activator of which the main is PAI-1. PAI-1 activity-neutralizing factors are XIIa, plasma kallikrein, and XIa [1] (Fig. 1).

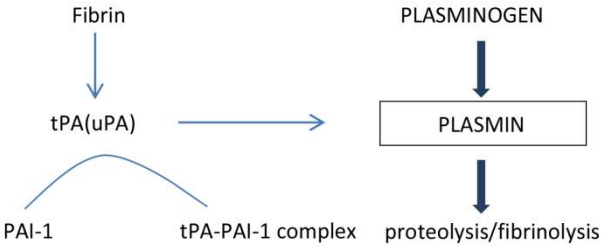

Fig. 1 Schematic presentation of activation of plasminogen

Roles of PAI-1

In addition to the role of antiprotease, PAI-1 has a high binding affinity for ligands of different receptors. Thus it affects cellular functions such as adhesion, migration, proliferation and intracellular signaling mechanisms that are similar in the cardiac muscle and renal mesangial

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matrix. Changes in the level of PAI-1 are strictly connected to the traditional and non-traditional risk factors and their complications. As an indication of the acute phase of inflammation or the acute inflammatory response, as well as metabolic control and neurohormonal activation, PAI-1 independently or synergistically with other pro-inflammatory molecule is a predictor of cardiovascular risk. In the literature it is described that a high level of PAI-1 is related to atherosclerosis and increased risk for plaque rupture [2]. In contrast to such findings, there are data from some researchers that there is profibrotic effect of PAI-1 in the affected muscles infarction, but also antifibrotic effect in the aging process.

Fibrinolytic System and Heart Disease

Decreased fibrinolysis with an increased concentration of PAI-1: tPA preformed complex PAI-1 in plasma, occurs in patients with coronary heart disease. The highest expression in the myocardium According to the type of circadian rhythm, the highest expression of PAI-1 in the myocardium is in the early morning hours at the exact time when an acute coronary syndrome exhibits a maximum incidence. Locally elevated PAI-1 predisposes the occurrence of the acellular thin atherosclerotic plaque attaching to the wall of the blood vessel and increasing the risk of rupture. When there is a rupture of PAI-1, it is released from the damaged plaque and from the platelet and local concentration of PAI-1 is increased by more than 10 times and a clot is formed. In recent decades, there have been developed percutaneous techniques for the repair of the stenosis of coronary blood vessels (including angioplasty, atherectomy, and stent implantation). Restenosis is associated with different supporting mechanisms involving different hemostatic factors and there is a slow increase in the concentration of PAI-1[3]. On the other hand, TAFI (thrombin activatable fibrinolysis inhibitor) is an antifibrinolytic factor whose growth increases with the influence of procoagulant stimulus but with contradictory research results concerning its association with myocardial infarction. It is clear that there is a direct molecular bond between the TAFI system with coagulation and fibrinolytic cascade [4]. However, the initiation of coagulation under the control of a sound is performed by the endothelial release of TFPI (tissue factor pathway inhibitor) which limits the activity of the TF / VIIa / Xa [5]. The determination of high levels of TAFI and low levels of PAI-1 prior to intervention is important to identify patients at high risk for restenosis. Increased number of late stent thrombosis is explained by the mechanical factor such as a posture in a stent in the penetration of the necrotic part of the tissue, biological factors such as the altered phenotype of endothelial cells and gene polymorphism. Genetic polymorphism explains the relationship between PAI-1 and risk of coronary heart disease. PAI-1 is synthesized and released via the sympathetic-adrenal axis for which it is the key mediator in the stress-induced thrombosis and hypercoagulability conditions [6] (Fig. 2).

Fibrinolytic System and Inflammation

The complex relationship between inflammation and thrombosis influences the course of coronary artery disease in metabolic syndrome. Proinflammatory particles stimulate multiple prothrombotic effects and acute coronary syndrome. Cardiovascular diseases with altered fibrinolytic system are connected to a raised level of PAI-1. Many authors emphasize the special role of the fibrinolytic system disfunction in metabolic syndrome related to the mechanism of coronary heart disease. The changing role of the fibrinolytic system is in connection with insulin resistance and visceral obesity, where PAI-1 plays a crucial role and its concentration rises in the blood and coronary plaque in these patients. The most common disorder of hemostasis, which occurs in these patients is reduced by the level of tissue factor pathway inhibitor, an elevated level of TAFI, VWF, fibrinogen, Factor VII, VIII and XIII and the vitamin K dependent coagulation proteins. Many authors recognize the bidirectional interaction between the elements of the metabolic syndrome and the expression of PAI-1. Adipocyte differentiation in the metabolic syndrome is induced by a set of signaling mechanisms that increase the expression of PAI-1 receptor and interfering with the signaling mechanisms of the insulin, and thus stimulate the formation of obesity [7]. It is assumed that the role of PAI-1 in metabolic syndrome is more important. Therefore, it is attractive as a target for developing new drugs. It has been shown that in the basis of atherosclerosis stands chronic inflammation, and that C-reactive protein (CRP) directly promotes the formation of atherosclerotic lesion through the interaction of the monocyte and endothelial cells by increasing the activity of PAI-1. CRP also increases the activity of PAI-1 in the state of hyperglycemia. On the other hand, CRP reduces the activity of the tissue PA in human endothelial cells which shows that CRP stimulates procoagulant effect (Fig. 3). CRP via activation of the protein kinase and

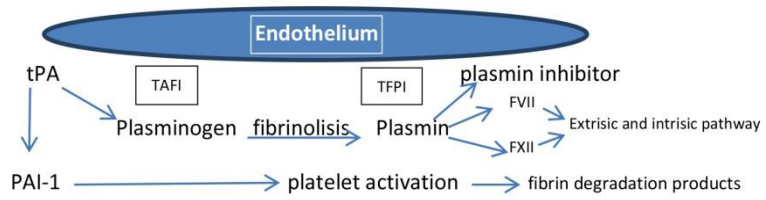

Fig. 2. Schematic presentation of the process of fibrinolysis

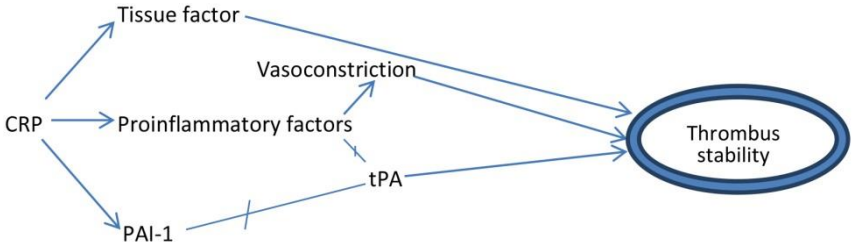

Fig. 3 Schematic presentation of the role of inflammation in the blood vessel reocclusion

NF-kB affects the expression of PAI-1 and affects hemodynamic induced migration of smooth muscle cells in blood vessels [8].

Apart from the acute phase, PAI-1 is increased in conditions of chronic inflammation, which is associated with renal disease. This partly explains the development of accelerated atherosclerosis in this patient population. Kidney diseases in which intra-renal expressions of PAI-1 are increased, are: diabetic nephropathy, focal segmental glomerulosclerosis (FSGS), membranous nephropathy, chronic allograft nephropathy, thrombotic microangiopathy, arteriolonephrosclerosis, "Crescent" focal necrotizing glomerulonephritis, glomerulonephritis. The level of PAI-1 is correlated with the severity of disease and in chronic renal disease, it accumulates in the interstitium [9].

Fybrinolitic System and Kidney Disease

It is believed that PAI-1 plays an important role in the complex mechanisms of tubulointerstitial renal fibrosis. Polymorphism of uric acid affects the plasma concentrations of PAI-1 and is linked with insulin resistance, level of circulating PAI-1 and activation of RAA system. In addition to genetic predisposition, factors which directly influence the production of PAI-1 are: glucose, insulin and various neurohumoral factors. Numerous studies have demonstrated an important role of PAI-1 in the development of diabetic complications [10]. Thrombin stimulates smooth muscle cells in blood vessels that release large amounts of PAI-1. In addition, it is known that the expression of the PAI-1 gene product is closely related to the differentiation of adipocytes and the phenotypic change of smooth muscle cells blood vessels. It is accepted that the PAI-1 risk factor for cardiovascular events in diseases is related with "bad" habits and lifestyle. Fibrosis is important for the development of chronic kidney disease. Decreased renal functional reserve leads to increased activity of inflammation and endothelial dysfunction. These processes increase the risk of the occurrence of cardiovascular disease and venous thrombosis and are associated with elevated levels of PAI-1. Decrease of *glomerular filtration rate* (*GFR*) increases the level of molecules which are markers of hemostasis. In addition, impaired renal function contributes to the formation of prothrombotic condition indirectly through the electrolyte disturbances and acid-base status influencing the change of activity of the enzyme coagulation [11]. It was found that atherosclerosis and risk of cardiovascular disease are associated with inflammation and procoagulant state. Patients with GFR <60 ml/ min/1.73m^2 have 6.5% higher levels of PAI-1 in comparison to subjects with GFR> 90ml/min/1.73m^2 which indicates that a disturbance homeostasis plays an important role in chronic kidney disease [12]. The fifth stage of chronic kidney disease has a three times higher risk for cardiovascular events compared with the general population while patients on chronic hemodialysis have up to 100 times higher risk of cardiovascular mortality compared with the general population younger than 45 years. Biomarkers of inflammation and oxidative stress are predictors of cardiovascular events in patients on chronic hemodialysis. During hemodialysis, blood in contact with a dialyser activates kallikrein kinin system and induces an inflammatory response by activating leukocytes and the production of cytokines [13]. Cytokines such as TNF alpha, IL 1 beta and IL 6 stimulate the expression of PAI-1 of a large physiological fibrinolysis inhibitor *in vivo* [14, 15]. It is well known that a continuous build-up of fibrin deposition in the renal transplant mechanism leads to chronic graft rejection. The mechanism of accumulation of fibrin, tPA is super expressed only in the acute phase of rejection. Upregulation of tPA is present during the progressive stages of chronic rejection and is synchronized with the induction of PAI-1 in the graft [16]. This means that the induction of PAI-1 is responsible for the accumulation of fibrin resulting in chronic irreversible damage and chronic kidney disease. Testing of PAI-1 genotype is in correlation with development of fibrosis, atrophy of tubules and interstitial fibrosis. It has been shown that genetic variation of fibrinolytic system affects long-term outcome of kidney transplantation. It has been found among people with previous acute rejection that G4 homozygous for the polymorphism had a significantly higher risk of kidney rejection. The conclusion of a number of studies is that determination of the genotype PAI-1 prior to transplantation could help to identify patients who are at risk for chronic rejection of transplanted kidney [17]. PAI-1 is a molecule, which is regulated not only genetically but also by metabolic and inflammatory factors, and it is known that the level of PAI-1 is correlated with the level of decreased function of the graft.

Conclusion

Determining prothrombotic biomarkers may help in the planning of timely and appropriate preventive measures for all patients who are at high risk of adverse cardiovascular events. During the last decade there has been an increase of interest in the introduction of non-invasive methods or biomarkers that would assess the degree of fibrosis in the kidney. In addition, experimental tests are underway of oral active PAI-1 inhibitor, which is a new class of anti-inflammatory agents.

References

1. Jiang Q, Gingles NA, Olivier MA, Miles LA, Parmer JR. The anti-fibrinolytic SERPIN, plasminogen activator inhibitor 1(PAI-1), is targeted to and released from catecholamine storage vesicles. Blood 2011; 117:7155-7163.
2. Xu Z, Francis J, Ploplos C, Ploplis VA. Plasminogenactivator inhibitor-1 (PAI-1) is cardioprotective in mice by maintaining microvascular integrity and cardiac architecture. Blood 2010; 115:2038-2204.
3. Ridker MP, Rifai N, Clearfield M, Downs JR, Weis ES, Miles S et al. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. N Engl J Med 2001; 344(26):1959-1965.
4. Milijic P, Willemse J, Djordjevic V, Radojkovic D, Colovic M, Elezovic I, et al. Thrombin Activatable Fibrinolysis Inhibitor (TAFI): A Molecular Link Between Coagulation and Fibrinolysis. Srp Arh Celok Lek 2010;138(1):74-78.
5. Van Hinsbergh WMV. Endothelium-role in regulation of coagulation and inflammation. Semin Thromb Hemost 2012; 34:93-106.
6. Flevaris P, Vaughan D. The role of plasminogen activator inhibitor type-1 in fibrosis. Semin Thromb Hemost 2017; 43(2):169-177.
7. Sharony R, Yu PJ, Park J, Galloway CA, Mignatti P, Pintucci G. Protein targets of inflammatory serine proteases and cardiovascular disease. J Inflammat 2010; 7:2-17.
8. Miles LA, Parmer M, Pamer JR. PAI-1: Cardiac friend or foe? Blood 2010; 115:1862-1863.
9. Pretorius M, Donahue BS, Yu Ch, Greelish PJ, Roden DM, Brown NJ. Plasminogen activator inhibitor-1 as a predictor of postoperative atrial fibrillation after cardiopulmonary bypass. Circulation 2007; 116(Suppl 1):1-7.
10. Hillenbrand A, Knippschild U, Weiss M, Schrezenmeier H, Henne-Bruns D, Huber-Lang M, et al. Sepsis induced changes of adipokines and cytokines - septic patients compared to morbidly obese patients. BMC Surgery 2010; 10:26.
11. Tasic D, Radenkovic S, Kocic G, Ilic Deljanin M, Ignjatovic A. Microinflammation Factors in the Common Diseases of Heart and Kidneys. Disease Markers 2015. Article ID 70589. http//dx.doi.org/10.1155/2015/470589.
12. Tasic D. Clinical modalities of the cardiorenal syndrome and the significance of certain biomarkers in its estimation [dissertation on the Internet]. Niš: Universitz of Niš, Faculty of Medicine; 2015. Available from: https://fedorani.ni.ac.rs/fedora/get/o:1027/bdef:Content/download [in Serbian]
13. Velickovic D, Djukic D, Tasic D. Nonsteroidal anti-inflammatory drugs and hypertension – does an exeption change the rule? Acta Medica Mediane 2014; 53(3): 25-31. doi:10.5633/amm.2014.0304
14. Devaraj S, Xy ZD, Jialal I. C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells: implications for the metabolic syndrome and atherothrombosis. Circulation 2003; 107:398-404.
15. Zeisberg M, Neilson G. Mechanisms of tubulointerstitial fibrosis. J Am Soc Nephrol 2010; 21:1819-1834.
16. Ichimura A, Matsumoto S, Suzuki S, Dan T, Yamaki S, Sato Y, et al. A small molecule inhibitor to plasminogen activator inhibitor 1 inhibits macrophage migration. Arterioscler Thromb Vasc Biol 2013; 33:935-942.
17. Fogo AB. Renal fibrosis: not just PAI-1 in the sky. J Clin Invest 2003; 112:326-328.

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Review Article

THE INFLUENCE OF VARIOUS MICROENVIRONMENTAL FACTORS ON BIOMECHANICAL FEATURES OF DIFFERENT SUTURE MATERIALS USED IN HEPATO-PANCREATO-BILIARY SURGERY

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Abstract. *The key features of any suture material, such as its tensile strength, knot security, resorbability, handling characteristics and biological behavior must be taken into account during the selection process. These biomechanical features may be variable in different microenvironmental conditions in the human body in which the sutures are placed due to the influence of numerous local biohumoral factors. We have reviewed the data on the impact of pancreatic juice and bile, various pH conditions, chemotherapy and heat on different suture materials behavior. It is suggested that in pancreatic and biliary surgery polydioxanone sutures should be used. The review has also demonstrated that absorbable suture materials were more sensitive to pH than non-absorbable sutures. In addition, polyglyconate sutures were the strongest of all absorbable synthetic sutures when exposed to heat and cytotoxic drugs. This review provides a better basis for the selection of suture materials for specific applications.*

Key words: *tensile strength, suture materials, pancreatic juice, bile, pH condition, chemotherapy*

Introduction

Surgical sutures are among the most commonly used medical devices. Their design and composition, along with the application technique, are essential for successful wound healing [1]. Although many issues are involved in the wound healing process special consideration should be taken with regard to the exposure of the suture materials to various microenvironmental biohumoral factors in the different tissues. These include different body fluids, pH conditions, local temperature etc.

Types of suture materials and their classifications are presented in Table 1. The most common classification of surgical suture materials is based in their resorbability and includes absorbable (AB) or non-absorbable (NAB) sutures. Also, based on their composition within these two categories, there are three types of materials used: natural unmodified, natural modified, and synthetic. In addition, based on their structure they are divided to monofilament and multifilament, coated or uncoated, with straight or curved needle. Furthermore, sutures can be classified according to their sizes as well as to the method of application, The performances of the suture material are adjusted to the specific tissue- and microenvironment-related conditions and demands, so the sutures are also classified for particular exclusive application in different types of surgery. The key features of any suture material, such as its tensile strength, knot security, resorbability, handling characteristics and biological behavior must be taken into account during the selection process [2].

Possible mechanisms underlying the changes of the suture features in different conditions will be discussed below, with an emphasis on pancreatic juice and bile, various pH conditions, chemotherapy and heat. Furthermore, these changes are also important considering that some of these materials are nowadays being widely investigated as possible constituents of some more complex prosthetic devices.

Biomechanical properties of the suture material

The biomechanical properties of the suture are of major concern to surgeons. The capacity of a suture to withstand the tension forces that lead to its stretching and rupture is defined as tensile strength. It is under the direct influence of numerous factors, most important being the material components, the cross-sectional area thickness and the stretching force. In terms of surgical practice, tensile strength is the time it takes for suture to lose 70% to 80% of its initial strength. Most available AB sutures halve their strength within 4 weeks. However, lactide-based copolymer AB sutures can maintain the strength over the period of 2 months. For most of the AB sutures, required full absorption time ranges from several months to years [1]. It is important to notice that

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the loss of tensile strength and mass absorption are two separate processes. Although the suture loses its strength much faster than it is absorbed, it must support the wound long enough to enable healing after which it remains in the tissue as a foreign body for a much longer period [2].

In the early stages of wound apposition and healing, high tensile strength of sutures is important, while retention of strength is important in delayed wound healing situations [3]. Suture strength is influenced by various conditions prevailing in a particular part of the body. In many areas the changes of the fiber properties

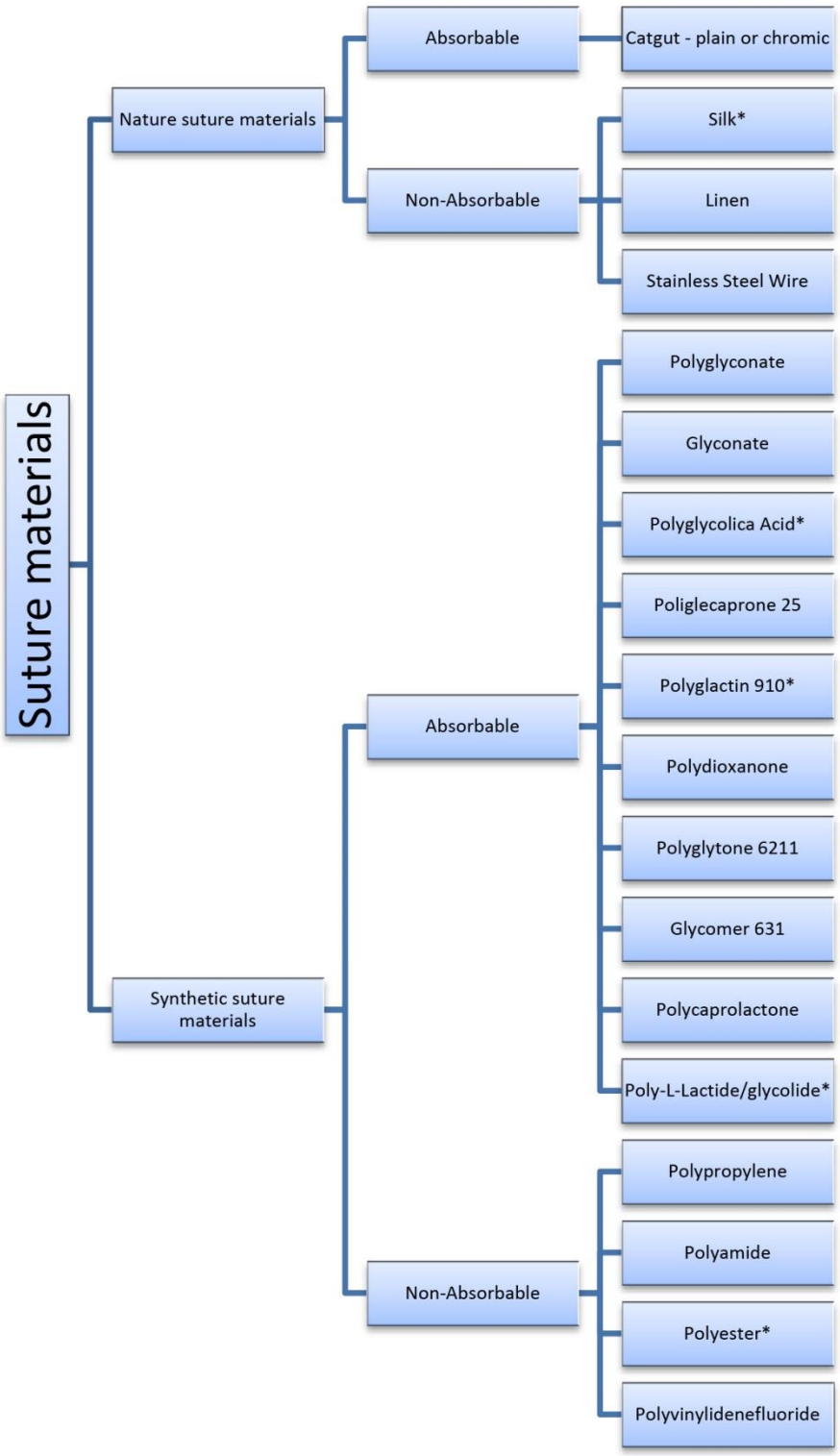

Fig. 1 Different types of suture materials with their classifications (natural and synthetic; absorbable and non-absorbable; mono-filament and multi-filament).
* Multi-filament (braided) sutures, whereas all other unmarked sutures (without *) are mono-filament.

and weakening of the tensile strength occur due to the influence of various local biohumoral factors. Also, it is noted that the weakest point of a surgical suture is the knot, regardless of the suture material and the knot configuration [4, 5]. It is important to know the tensile strength of the suture and its changes in different pH environments [6, 7], under the influence of body fluids [8, 9], or due to the presence of bacterial adherence [10]. These changes may irreversibly alter the suture dynamics [11], knotting characteristics [12, 13] and biocompatibility [14], and eventually lead to impaired healing.

Effect of pancreatic juice and bile on the tensile strength of suture materials

It is assumed that pancreatico-jejunostomy has a higher failure rate compared to other surgical anastomoses. One of the reasons for that may be the exposure of the sutures to pancreatic juice. Specific effects of pancreatic juice and bile on the tensile strength of suture materials derive from the composition of these two body fluids. Bile contains water, electrolytes, bile salts, proteins, lipids, and bile pigments, and it is an alkaline fluid. Pancreatic fluid contains approximately fifteen enzymes or precursors of enzymes [8].

There is little data on the effects of pancreatic juice and bile on suture materials. The aim of the study by Muftuoglu et al. (2004) was to observe such effects on absorbable and non-absorbable suture materials [8]. The authors suggested that the application of catgut and silk should be avoided and for mucosal layer of pancreaticojejunostomy non-absorbable sutures should be used. This research also showed that PGA sutures and polyglactin 910 were not appropriate for use in pancreatic surgery. Furthermore, the authors proposed PDO sutures for application in pancreas surgery because of their resistance to pancreatic fluid and lesser associated inflammatory reaction [8]. Biliary and pancreatic surgery are associated with increased risk of fistula [8]. The nature of the suture used may have a role in the genesis of pancreatic fistula, in addition to multiple factors involved in the development of this complication [9]. The majority of absorbable suture materials are sensitive to proteolytic enzymes contained in pancreatic juice because they are made of proteins [9].

One of the earliest researches was the study by Mizuma et al. investigating the changes of loop-tensile strength of various suture materials when exposed *in vitro* to pancreatic juice [9]. It was concluded that due to the exposure to pancreatic juices and consequent proteolysis, 24-48 hours after surgery, catgut could not maintain its tensile strength and could not hold the pancreatic duct and jejunum connected. Furthermore, their research demonstrated that catgut sutures were disintegrated even when almost all trypsin activity was inhibited and this was due to the presence of other proteolytic enzymes in the pancreatic juice that may be involved in the process of digestion. Also, non-activated pancreatic juice was capable of digesting the catgut because these pancreatic enzymes were active at least partially in the form in which they were secreted [9]. Interestingly, the finding that the addition of aprotinin has a protective effect might have practical significance [9].

According to the results of Mizuma et al., PGA sutures maintained their strength better than catgut, but not as well as silk or nylon. Also, because of their structure, polyglycolic sutures were digested by non-activated pancreatic juice faster than by activated pancreatic juice [9]. Furthermore, in numerous studies it was concluded that synthetic suture materials were better for use in pancreas surgery [15, 16].

Effect of various pH conditions on the tensile strength of suture materials

It is very important for the surgeon to know how the strength of suture materials is influenced by the different chemical components and features of the body fluid in direct contact with the thread, particularly the pH level, after their implantation. In different circumstances, especially under pathologic conditions, the pH of body fluids may alter the biomechanical characteristics of the suture. Thus, a study of the pH effect on tensile strength of sutures will provide a better basis for the selection of suture materials for specific applications.

Under normal circumstances, the pH of gastric juice varies from 0.9 to 1.5 while pancreatic juice in the duodenum ranges from 7.5 to 8.2 and urinary pH ranges from 4.5 to 8.0. Chu's et al. categorized and summarized the effects of pH on eight different AB and NAB suture materials, based on an *in vitro* immersion study. The reported pH dependent degradation of sutures in this study deserves the attention of surgeons in their selection of these suture materials in various physiologic and pathologic conditions. Furthermore, in this study it was indicated that AB suture materials were more sensitive to pH than NAB suture materials [17].

Tomihata et al. showed that sutures containing glycolic acid as a comonomer were degraded faster in alkaline solution, whereas PDO sutures had a faster degradation in acidic solution [18]. In addition, Chu et al. (1983) demonstrated that both acidic and alkaline environments might accelerate the degradation of natural AB sutures, while only alkaline conditions had this undesirable effect on synthetic AB sutures. This observed pH dependence of PGA and polyglactin 910 sutures was consistent with the previous reports [19, 20], but it was contrary to Holm-Jensen and Agner's and Reed and Gilding's data [21, 22]. It is hypothesized that the coating materials and/or the level of crystallinity may contribute to this discrepancy. In the same investigation, within the NAB sutures, silk was the most sensitive in various pH conditions. Polyamide [multifilament) sutures were the next most sensitive to pH levels, while polyamide (mono-filament), polyester, and polypropylene sutures had very similar pH dependence [17].

Many authors [7,23] were consistent with results of Chu's et al. (1983) research indicating that accelerated

loss of strength was present in both acidic and alkaline environments. Also, it is claimed that the suture implantation site, which is always presented with inflammation, is generally on the acidic side of the pH scale [17]. This may have a major importance in certain situations. For example, the transfixion of a bleeding ulcer in the environment with low pH due to the presence of HCl should not be performed with plain catgut sutures. NAB sutures that maintain their tensile strength for 4 weeks are the right choice in these situations. For closure of the intestines the choice should be the AB sutures in order to avoid the narrowing of the intestinal lumen and bacterial migration along the fibers [17].

Preservation of adequate strength in pancreatic juice is especially important. In Chu's study it was noted that polypropylene, polyester, and polyamide (mono-filament) were the NAB sutures of choice rather than silk sutures and polyamide (multifilament), while polyglactin 910 was the best among the AB sutures. As for the urinary tract the AB suture is a better choice than NAB suture [17].

The study by Karabulut et al. (2010) aimed to investigate the effects of pH and *in vivo* and *in vitro* effects of different intra-abdominal organs and fluids on the retention of tensile strength of seven different suture materials. They summarized that polypropylene suture preserves its stability in all conditions and pH values. In addition, it is concluded that in urological surgery polyglytone 6211 and glycomer 631 should be used. In biliary surgery polyglactin 910, polyglyconate or glycomer 631 had the best performances, while the use of polypropylene, as the material with highest durability, was disputed because it is an NAB suture and could cause biliary stone formation [7].

Effect of chemotherapy and heat on the tensile strength of suture materials

The absorption rate and therefore the tensile properties of AB sutures are dependent on their chemical properties. There is numerous evidence that free radicals [24], enzymes [25], temperature [26], and pH [7] may affect the hydrolysis rate of glycolic and lactic acids polymers used in AB sutures structure. Because most of the chemical reactions increase with temperature rise, temperature is particularly important among these factors.
The process of tissue repair depends on both the surgical technique and the properties of the sutures used, as well as on tissue integrity. It is noted that impaired wound healing induced by chemotherapy agents and/or hyperthermia after hyperthermic intraperitoneal chemotherapy (HIPEC) procedures has been associated with bowel complications observed [5].

Lapointe's et al. (2016) study described for the first time the impact of heated chemotherapy on biomechanical properties of the commonly used gastrointestinal sutures. Thus, the objective of this study was to compare tensile breaking force and elongation rate of six different AB sutures, when exposed to heat and cytotoxic drugs: oxaliplatin and mitomycin-c, for a better understanding of their impact on gastrointestinal anastomosis. They concluded that tensile strength of all tested AB sutures was preserved for a minimum of 2 weeks. In addition, it was suggested that exposition to heat and chemotherapy did not significantly affect the biomechanical properties of tested sutures. However, this study did not analyze the long-term impact of heat and chemotherapy on sutures' properties. This study model was not sufficient for the final conclusion about the influence of suture choice on anastomotic leakage [5]. The selected heat model in this study included the temperatures of 37ºC and 45ºC, because of the increased risk of small bowel toxicity when intra-abdominal temperature is over 45ºC [15], while 37ºC mimics the body temperature. So far there is no clear position on the choice of suture materials for the use in cytoreductive HIPEC procedures. Attitudes applied by surgeons nowadays are based on individual experience [5].

Beside the tensile strength, another important biomechanical feature of the suture materials is elongation rate which is determined by both elasticity and structural properties of the suture material. The elongation rate of suture materials determines the suture adjustment to both wound edema and wound contraction. As showed in Lapointe's et al. (2016) paper, monofilament sutures had a significantly higher elongation rate than multifilament sutures in all experimental conditions [5]. Furthermore, Tomihata et al. (2005) noted that monofilament sutures provide higher elongation rate than braided sutures [27]. This is explained by the structural characteristics of monofilament sutures, i.e. more homogenous tubular structure which contributes to the higher elongation rate [28,29]. It is important to point out that some studies reported no correlation between the suture tensile strength and its elongation rate [5,30]. Furthermore, increasing the size of suture material increases its tensile strength.

Lapointe et al. (2016) showed in their study that under basal condition polyglyconate was the strongest of all tested AB synthetic sutures while tensile strength was like other AB sutures investigated [5]. However, Pietrzak et al. showed that AB polymers used in suture composition were affected by temperature [26]. The authors analyzed the impact of temperature on hydrolysis of a PGA/polylactic acid copolymer. Their results showed that the rate of hydrolysis increased with the rise of temperature. The interesting observation in these data was that a variance of as little as 2ºC from 37ºC could affect the rate of hydrolysis for about 25-30% [26]. This adverse finding in relation to the results by Lapointe et al. may be explained by the difference in incubation time.

In addition to the temperature, it should be taken into account that the pH of the incubating solution is not a less important factor that can affect hydrolysis rate of the tested AB suture. However, the whole experimental model in the research conducted by Lapointe et al. would have different results if it was implemented under

in vivo conditions [5], and therefore this is one of the main limitations of this study. Thus, these data must be interpreted with caution. Also, in an investigation by Pietzark et al. all animal testing needs to be interpreted with caution because most of them have a body temperature 1-3ºC greater than that of humans [26]. It may be that these variations in body temperature may contribute to faster tensile strength loss in animals. More research is required to determine the efficacy of chemotherapy and heat on the tensile strength of suture materials.

One should bear in mind that this part of the review is based on a small amount of the published data. Further studies need to be carried out in order to validate these adverse findings and determine a definitive statement on the effect of chemotherapy and heat on the tensile strength of suture materials.

Sutures and infection

Although not the primary goal of this review, the influence of bacterial accumulation and infection on the biomechanical features of surgical sutures should be mentioned. It is now well known that surgical sutures may serve as a favorable substrate for bacterial colonization and growth [31]. This bacterial colonization induces a hypoxic environment and inhibits the activity of fibroblasts and granulocytes, thus leading to the impaired host-defense response. This is followed by the creation of biofilm consisting of bacteria encapsulated within a self-produced extracellular polymeric matrix composed of polysaccharides, proteins and nucleic acids which serves as a self-protection for bacteria [32, 33]. Susceptibility of suture materials for bacterial adherence depends on their biomechanical and chemical features. For example, it is widely accepted that braided sutures are more at risk of contamination due to their larger scabrous surface that facilitates bacterial adhesion in comparison with monofilament sutures. In turn, bacterial colonization and especially infection significantly diminish the quality features of sutures including, but not limited to their tensile strength. Therefore sutures may also be graded by the level of their infectibility. In terms of their resistance to infection, synthetic and monofilament sutures are suggested as superior than natural and multifilament ones. However, as this is the field of wide scientific interest numerous data on this topic would exceed the scope of this review.

Conclusion

The present review was designed to determine the effects of different conditions on tensile strength of suture materials. It is suggested that in pancreatic and biliary surgery PDO should be used; for mucosal layer of pancreatic anastomosis, non-absorbable sutures were sutures of choice. The review has also demonstrated that absorbable suture materials were more sensitive to pH than non-absorbable sutures. Both acidic and alkaline environments might accelerate the degradation of natural absorbable sutures while only alkaline conditions had this undesirable effect on synthetic absorbable sutures. This review has found that polyglyconate was the strongest of all tested absorbable synthetic sutures when exposed to heat and cytotoxic drugs. The current data highlight the importance of different conditions on biomechanical features of suture materials. The review is limited by the lack of published information on this issue. It is suggested that the association of these factors should be more intensely investigated in future studies.

References

1. Ingram D. Bioswellable amphiphilic copolymers, MSc thesis. Clemson University: South Carolina, USA, 2012.
2. Thomas WEG. Sutures, ligature materials and staples. Surgery 2002; 20(5):97-99.
3. Field JR, Stanley RM. Suture characteristics following incubation in synovial fluid or phosphate buffered saline. Injury 2004; 35(3): 243-248.
4. Silver E, Wu R, Grady J, Song L. Knot security - how is it affected by suture technique, material, size, and number of throws? J Oral Maxillofac Surg 2016; 74:1304-1312.
5. Lapointe S, Zhim F, Sidéris L, Drolet P, Célestin-Noël S, Dubé P. Effect of chemotherapy and heat on biomechanical properties of absorbable sutures. J Surg Res 2016; 200(1):59-65.
6. Chung E, McPherson N, Grant A. Tensile strength of absorbable suture materials: in vitro analysis of the effects of pH and bacteria. J Surg Educ 2009; 66(4):208-211.
7. Karabulut R, Sonmez K, Turkyilmaz Z, Bagbanci B, Basaklar AC, Kale N. An in vitro and in vivo evaluation of tensile strength and durability of seven suture materials in various pH and different conditions: an experimental study in rats. Indian J Surg 2010; 72(5):386-390.
8. Muftuoglu MT, Ozkan E, Saglam A. Effect of human pancreatic juice and bile on the tensile strength of suture materials. Am J Surg 2004; 188(2):200-203.
9. Mizuma K, Lee PC, Howard JM. The disintegration of surgical sutures on exposure to pancreatic juice. Ann Surg 1977; 186(6): 718.
10. Masini BD, Stinner DJ, Waterman SM, Wenke JC. Bacterial adherence to suture materials. J Surg Educ 2011; 68(2):101-104.
11. Visser JD. Dynamic strength of surgical suture materials. in: Winter GD, Leray JL, deGroot K. (eds): Evaluation of biomaterials. Wiley, London, 1980.
12. Tera H, Aberg C. Tensile strengths of twelve types of knot employed in surgery, using different suture materials. Acta Chir Scand 1975; 142(1):1-7.
13. Stone IK, Masterson BJ, Von Fraunhofer JA. Knot stability and tensile strength of an absorbable suture material. Surf Coat Tech 1986; 27(3):287-293.
14. Stillman RM. Wound closure: choosing optimal materials and methods. ER Reports 1981; 2: 41-44.
15. Merei I, Nahm C, Ofri A, Samra J, Clarke E, Anubhav M. A Comparative in vitro assessment of suture strength after exposure to pancreatic enzyme solution. JOP. J Pancreas (Online) 2019; 20(5):138-141.
16. Karaman K, Bal A, Aziret M, Ercan M, Bostanci EB, Akoglu M. Which suture material is optimal for pancreaticojejunostomy anastomosis? An in vitro study. J Invest Surg 2017;30(4):277-284.
17. Chu CC, Moncrief G. An in vitro evaluation of the stability of mechanical properties of surgical suture materials in various pH conditions. Ann Surg 1983; 198(2):223-228.

18. Tomihata K, Suzuki M, Ikada Y. The pH dependence of monofilament sutures on hydrolytic degradation. J Biomed Mater Res 2001; 58(5):511-518.
19. Chu CC. A comparison of the effect of pH on the biodegradation of two synthetic absorbable sutures. Ann Surg 1982; 195(1):55.
20. Chu CC. The effect of pH on the in vitro degradation of poly (glycolide lactide) copolymer absorbable sutures. J Biomed Mater Res 1982; 16(2):117-124.
21. Holm-Jensen S, Agner E. Syntetisk absorberbart suturmateriale (PGA) sammenlignet med catgut. Ugeskrift Laeger 1974; 136(32):1785-1790.
22. Reed AM, Gilding DK. Biodegradable polymers for use in surgery - poly (glycolic)/poly (lactic acid) homo and copolymers: 2. In vitro degradation. Polymer 1981; 22(4):494-498.
23. Abellán D, Nart J, Pascual A, Cohen RE, Sanz-Moliner JD. Physical and mechanical evaluation of five suture materials on three knot configurations: an in vitro study. Polymers 2016; 8(4):147.
24. Lee KH, Chu CC. The role of superoxide ions in the degradation of synthetic absorbable sutures. J Biomed Mater Res 2000; 49(1): 25-35.
25. Williams DF, Mort E. Enzyme-accelerated hydrolysis of polyglycolic acid. J Bioeng 1977; 1(3):231-238.
26. Pietrzak WS, Kumar M, Eppley BL. The influence of temperature on the degradation rate of LactoSorb copolymer. J Craniofac Surg 2003; 14(2):176-183.
27. Tomihata K., Suzuki M, Tomita N. Handling characteristics of poly (L-lactide-co-ε-caprolactone) monofilament suture. Biomed Mater Eng 2005, 15(5):381-391.
28. Kim JC, Lee YK, Lim BS, Rhee SH, Yang HC. Comparison of tensile and knot security properties of surgical sutures. J Mater Sci Mater Med 2007; 18(12):2363-2369.
29. Türker M, Kılıçoğlu Ö, Salduz A, Bozdağ E, Sünbüloğlu E. Loop security and tensile properties of polyblend and traditional suture materials. Knee Surg Sports Traumatol Arthrosc 2011; 19(2):296-302.
30. Freudenberg S, Rewerk S, Kaess M, Weiss C, Dorn-Beinecke A, Post S. Biodegradation of absorbable sutures in body fluids and pH buffers. Eur Surg Res 2004; 36(6):376-385.
31. Henry-Stanley MJ, Hess DJ, Barnes AM, Dunny GM, Wells CL. Bacterial contamination of surgical suture resembles a biofilm. Surg Infect 2010;11:433-439.
32. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2004;2:95-108.
33. Fux C, Costerton J, Stewart P, Stoodley P. Survival strategies of infectious biofilms. Trends Microbiol 2005;13:34-40.

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Case Report

SECONDARY ADRENAL INSUFFICIENCY MIMICKING ABDOMINAL CAUSE OF RECURRENT ABDOMINAL PAIN IN A PREPUBERTAL GIRL

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Abstract. *Recurrent abdominal pain (RAP) in children is defined as at least three episodes of pain that occur over at least three months and affect the child's ability to perform normal activities. The prevalence of recurrent abdominal pain in a population of schoolchildren varies from 10% to even 45%. Pituitary disorders are rarely considered as causative factor in children with abdominal pain, especially in absence of midline facial defects and in case of emergency, when growth monitoring is neglected or not performed carefully. Herein we report an unusual case of RAP caused by secondary adrenal insufficiency. An 8-year-old girl was hospitalized repeatedly because of RAP. Biological parameters including white blood cell count, hemoglobin value, C-reactive protein, glucose, electrolytes were within the normal range. Low cortisol combined with low ACTH values was the clue for diagnosis. The magnetic resonance imaging (MRI) of the pituitary region revealed anterior pituitary hypoplasia and pituitary stalk interruption. To the best of our knowledge, this is the first report of child with RAP caused by pituitary disorder. Possible inherited, autoimmune and post-traumatic causative factors are discussed.*

Key words: *recurent abdominal pain, pituitary hypoplasia, secondary adrenal insufficiency, growth hormone deficiency, hypopituitarism, pituitary stalk lesion*

Introduction

Recurrent abdominal pain (RAP) in children is defined as at least three episodes of pain that occur over at least three months and affect the child's ability to perform normal activities [1]. It is one of the commonest reasons for referral to pediatrician and pediatric gastroenterologist, as well to pediatric surgeon. Almost all children have abdominal pain at one time or another. The prevalence of recurrent abdominal pain in a population of schoolchildren varies from 10% to even 45% [1, 2]. Mostly, it is not caused by a serious medical problem, and has transient nature. However, a vast majority of abdominal and non-abdominal causes could be involved, classified as organic or non-organic (functional, like "visceral hyperalgesia") [3]. It is noteworthy to say that abdominal pain can be a sign of serious illness that seeks urgent and specific treatment. We here report an unusual case of recurrent abdominal pain accompanied by growth failure , fatigue, weakness and weight loss, caused by endocrine disorder: pituitary-adrenal axis insufficiency.

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Case Report

Initial examination

In August 2018, an 8-year-old girl was referred to Pediatric Clinic of Nis because of acute abdominal pain with formed stools without hematochezia. Abdominal pain started ten days before admission. It was located in periumbilical region and was more prominent in the right iliac fossa, without vomiting and fever. Neither urinary disorders nor weight loss were documented on first admission. Her examination otherwise was normal.

She was born at term after an uneventful pregnancy; birth weight was 3.150 g (P25), birth length of 54.0 cm (P90) on Fenton growth charts. Apgar score was not available, but spontaneous breathing without cyanosis or jaundice was documented. No dysmorphic features were noticed. She was breast fed from birth until the age of 10 months when gluten was introduced into the diet without any adverse gastrointestinal effect. At the age of 3 years she experienced head injury, estimated as non-serious. Both parents were healthy and unrelated, with no history of endocrine or autoimmune diseases and with normal pubertal development.

She was proportionally short; her height of 121cm was -1.88 SD (P3) for her Chronological Age (CA), her Height Age (HA) corresponded to 7 years, and her Body Mass (BM) of 22 kg was 0.6 kg below her ideal weight-for-height. Her BMI was 21.5 kg/m2 (P27). Bone age was retarded and corresponded to 5.5 years. No signs of puberty were registered. Growth records before 8 years of age were unavailable.

A pediatric surgeon reviewed and observed her for three days. A physical examination revealed a distended abdomen without defense or masses. Bowel sounds were present without organomegaly. An X-ray of abdomen showed only signs of constipation. The abdominal ultrasound (US) was reported as normal. Conclusion: no surgical cause of abdominal pain was discovered.

First hospitalization

An investigation of pediatric gastroenterologist was indicated. The girl was referred again to Pediatric Clinic for further investigation. Complete systemic examination showed no pathological findings. Biological parameters including white blood cell count, hemoglobin value, C-reactive protein, glucose, electrolytes were within the normal range. Stool cultures were negative. Viral tests (CMV, EBV, HSV1, and Coxsackie virus) were also negative. Furthermore, serum anti-tissue transglutaminase (tTG) antibodies (immunoglobulin IgA-tTG = 0.1 U/mL, normal values <10 U/mL), ASCA IgA and IgG were negative. Fecal calprotectin test was within normal range. MSCT of abdomen and pelvis was performed and it came back normal. During hospitalization she also complained of headaches without vomiting. An examination of pediatric neurologist showed no structural causes identified of present headaches. She was treated by conservative management (rehydration and analgesia). Conclusion: no structural cause identified of abdominal pain.

The patient was discharged from the hospital with advice to use antispasmodic therapy in abdominal pain episodes.

Second hospitalization

Two weeks later, the girl was urgently admitted to Pediatric Clinic due to the worsening of symptoms. On examination, she complained of severe abdominal pain located again periumbilically and more to the right iliac fossa. A few days before admission, she was febrile to 39^0 C. The pain occurred every day in the afternoon or early evening with vomiting. She had regular stools. Weight loss of about 3.5 kg was reported during more than a month. No history of polydipsia, polyuria and heat/cold intolerance was reported. She looked unwell and had clinical dehydration. Evaluation of her vital signs revealed slightly decreased blood pressure at 95/65mmHg and normal heart rate to 87/min. Her respiratory rate was 26 breaths/min. The girl was awake and oriented but appeared weak and fatigued. Her skin turgor was reduced and capillary refill time was 2 seconds. In the right frontal part of the scalp we noticed a white scar-like linear change of 2.5 cm in length. The neck was supple with no goiter or adenopathy. The lungs were clear to auscultation and the heart revealed no murmurs. Bowel sounds were audible with normal genitals and no deformities in extremities were observed.

Laboratory investigations were within the normal range including electrolytes. Blood film was reported as normal. Her urine was clear on dipstick testing; urinary microscopy and culture were negative. An abdominal ultrasound (US) was requested and was reported as normal. Initially, the girl was treated by conservative management (IV rehydration with crystalloids) and analgesia. During hospitalization, she received intravenous antibiotics and corticosteroids. A pediatric surgeon was consulted and he did not find any surgical cause of the abdominal pain. This time he advised endoscopic examination. Fecal calprotectin test was positive (1490 µg/g), but the control calprotectin value was within normal range (26.6 µg/g). Upper endoscopy and colonoscopy revealed normal bowel structure. MRI enterography was performed and was reported as normal without any sign of inflammatory bowel disease (IBD). Repeatedly, the gastrointestinal causes of abdominal pain were excluded. However, the clinical picture persisted despite adequate conservative management and analgesia. It is noteworthy to say that symptoms aggravated during afternoon.

What could be the cause of recurrent abdominal pain in our patient? We considered:

- Functional abdominal pain in which no structural causes are identified in 90% of cases
- Surgical disorders;
- Gastrointestinal disorders;
- Systemic disorders;
- Renal disorders;
- Hepatobiliary/pancreatic disorders;
- Neurological/Psychosocial disorders;
- Malignant diseases;
- Endocrine disorders
- Metabolic disorders - porphyria.

Then we focused our investigation on excluding possible endocrine causes of this abdominal pain. Despite the normal blood glucose and electrolytes, the endocrinologist suggested the possibility of adrenal insufficiency and advised examination of hormonal status including TSH, FT4, PRL, cortisol and ACTH.

Extremely low levels of cortisol (20nmol/l) and inappropriately low ACTH (1.8 pg/ml) were found, revealing secondary adrenal insufficiency (Table1). Levels of TSH, FT4, and PRL were within normal range. Repeated check of morning cortisol and ACTH level confirmed secondary adrenal insufficiency. Short ACTH stimulation test was not done because of lack of the test dose and even more because of the necessity of urgent substitution therapy.

Table 1 Confirmatory serum testing for secondary adrenal insufficiency

Test	Patient's result	Normal value
ACTH (pg/ml)	1.8pg/ml	(7.20-63.30)
Cortisol (nmol/l)	20.0 nmol/l	(166.0-507.0)
Cortisol in ACTH stimulation test	Non available	> 550

The magnetic resonance imaging (MRI) of the pituitary region showed the anterior pituitary hypoplasia and pituitary stalk interruption (Fig. 1 and Fig. 2). We concluded that the cause of adrenal insufficiency in this case is of pituitary origin: pituitary hypoplasia (congenital or acquired) which resulted in partial hypopituitarism, presenting as RAP caused by secondary adrenal insufficiency.

The patient's abdominal symptoms resolved immediately after introduction of the first doses of substitution therapy with higher physiological doses of hydrocortisone. Hydrocortisone is given in 3 separate doses with a typical total daily dose of 15mg/m²; 7.5 mg, in the morning, 2.5 mg at lunchtime and early evening (6:00PM). During acute febrile illness or in case of trauma, the parents were advised to double the hydrocortisone dose. In case of vomiting and diarrhea, the advice was to triple the daily dose. In the later occasion, it would be necessary to give intravenous hydrocortisone and crystalloids [4].

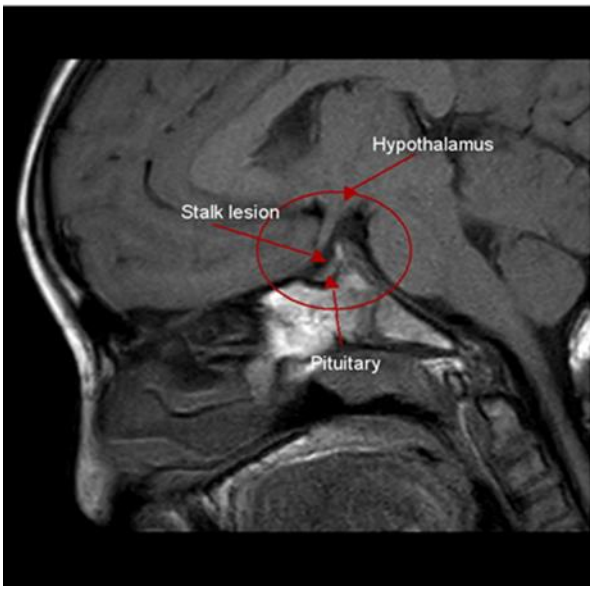

Fig. 1 Sagittal pituitary MRI showing pituitary hypoplasia

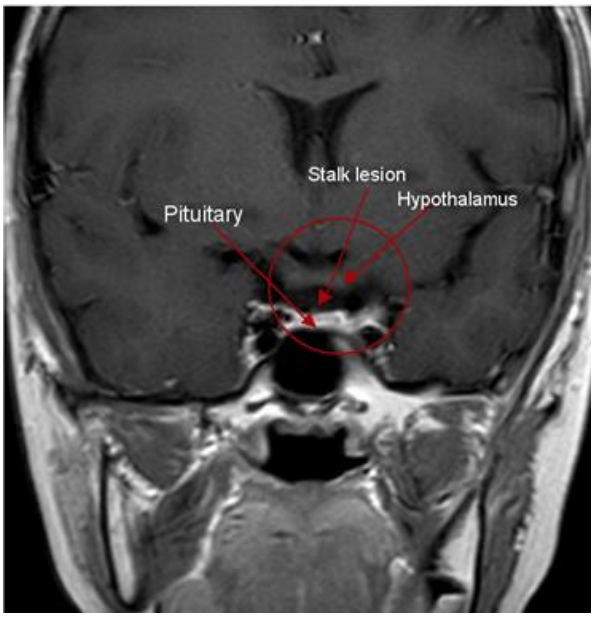

Fig. 2 Coronal pituitary MRI

Evaluation of the treatment effect

Two weeks later, the girl was admitted for evaluation of the replacement therapy quality. Her abdominal symptoms resolved with implementation of hydrocortisone, but the fatigue persisted. Hypotension and headache were still reported.

Control hormonal status showed still low values of morning cortisol and ACTH, low levels of PRL and euthyroid state. The patient's gonadotropin levels corresponded to prepubertal values. Hydrocortisone therapy was corrected and increased to a total daily dose of 18 mg/m²: 10mg in early morning, 5mg at lunchtime and 2.5 mg at early evening (6:00 PM), with advice of dose adjustment as above.

Since the patient's height of 120.5cm has been on P3 for her Chronological Age (CA) in discordance with MPH (Mean Predicted Height calculated being on P75) and the fact that some complaints could be attributed to growth hormone deficiency (GHD), recombinant growth hormone was introduced without previous testing of somatotropic axis. We made this decision based on documented structural pituitary stalk lesion and anterior pituitary hypoplasia. IGF1 determination was not available. Growth hormone deficiency was assumed as certain and under such circumstances GH stimulation tests are unnecessary, and even dangerous [5–11]. Introduction of GH therapy led to complete patient's recovery. Further follow-up and growth will eventually unmask other pituitary deficiencies.

Discussion

Pituitary disorders are rarely considered as a causative factor in children with abdominal pain, especially in absence of midline facial defects and in cases of emergency, when growth monitoring is neglected or not performed carefully. In our experience, pediatric gastroenterologists do care about neighboring organ status (testis torsion or basal pneumonia), but with the exception of DKA (Diabetic Ketoacidosis) or CAH (Congenital Adrenal Hyperplasia), they do not consider any other endocrine cause of abdominal pain (hypopituitarism, pheochromocytoma or hyperparathyreodismus i.e.). Herein we reported an unusual case of RAP caused by secondary adrenal insufficiency. Low cortisol combined with low ACTH values was of diagnostic value. Pituitary morphology investigation revealed structural abnormality of pituitary and pituitary stalk: anterior pituitary hypoplasia and pituitary stalk interruption (Fig. 1 and Fig. 2). Posterior pituitary was *in situ* with normal "bright reflex" on MRI (Fig. 1).

Adrenal insufficiency may result from a wide variety of congenital and acquired disorders of adrenal cortex, pituitary and hypothalamus (Table 2), adapted from Sasigarn and Rohan [12]. Destruction or dysfunction of the adrenal cortex is the cause of primary adrenal insufficiency (PAI) and its initial clinical presentation or relapse is frank and well known as "adrenal crisis" (AC), a medical emergency presenting as vomiting and abdominal complaints imitating gastrointestinal cause of dehydration. The progression could be gradual and nonspecific from hypotension to shock, depending on the degree of insufficiency and precipitating stress events. The electrolyte profile is typical; hyponatremia, hypochloremia, hyperkalemia (usually in PAI) and hypoglycemia (in children) accompanied by metabolic acidosis and occasionally hypercalcemia . However, it is very often misdiagnosed despite of well-established diagnostic criteria [13–18]. In contrast, electrolytes in secondary adrenal insufficiency (SAI) are usually within normal range. The hypoadrenalism is therefore due to pituitary or hypothalamic disorder, resulting in absence of the normal ACTH stimulation to the adrenal cortex. The consequence is partial or total cortisol deficiency but often a normal or near normal production of aldosterone. Mineralocorticoid secretion is not impaired because of primary regulation of aldosterone synthesis by the renin-angiotensin system among other stimuli. That is why it is more hidden and difficult to recognize. Cortisol provides some 30-50% of normal mineralocorticoid activity as it is present in great excess to aldosterone. The clinical symptoms of secondary adrenal insufficiency are also related to the degree of cortisol deficiency. The most common signs and symptoms are dry skin, decreased pubic/axillary hair, myalgia, arthralgia, severe fatigue, abdominal pain, loss of appetite, weight loss, nausea, vomiting, diarrhea, muscle weakness, headache, irritability, and depression or decreased consciousness. Since aldosterone is typically not diminished, low blood pressure and muscle spasms are not so prominent and severe as they are in primary adrenal insufficiency. Unlike in Addison's disease, hyperpigmentation does not occur [12, 15].

Pituitary stalk lesions (PSL) in children depicted on MRI investigation are rarely reported. There are only few clinical series published recently [19–21]. Secondary adrenal insufficiency was the least common condition and, when present, was always associated with deficiency of at least one other axis [19]. In our patient, documented secondary adrenal insufficiency and "certain"GH deficiency could be acquired as consequence of acute posttraumatic hypopituitarism (MRI of the pituitary region showed pituitary stalk interruption). Also, hypopituitarism could be congenital taking into account the MRI finding of the pituitary hypoplasia. Hamilton an al examined the causes of pituitary stalk lesions in adults and children. They noted that in adults inflammatory lesions were most common, while in children congenital lesions predominated [22]. Although, the MRI of the pituitary region in our patient showed only pituitary hypoplasia and pituitary stalk interruption, there were no criteria for Pituitary stalk interruption syndrome (PSIS). PSIS is characterized by MRI finding of a thin or absent pituitary stalk, associated hypoplastic or aplastic anterior pituitary and ectopic posterior pituitary (EPP). Clinically, PSIS can be associated with midline defects and various pituitary endocrine deficiencies, ranging from isolated growth hormone deficiency (IGHD) to combined pituitary hormone deficiency (CPHD). The endocrine outcome seems to be a progressive onset of hormone deficiencies leading to panhypopituitarism, but posterior pituitary function is usually maintained [22, 23].

The clinical presentation of posttraumatic stalk lesion can be similar as in PSIS [24]. Our patient does not fulfill MRI criteria for PSIS, since no ectopic posterior pituitary was found. It was challenging to find the cause of hypopituitarism; congenital pituitary hypoplasia due to genetic mutation, posttraumatic stalk lesion, lymphocytic hypophysitis or all together. Unfortunately, genetic analysis was not available, as well as pituitary antibodies determination [25]. The patient did have a history of the head injury. The hormonal axis most often chronically affected in patients with traumatic hypopituitarism

Table 2 Causes of adrenal insufficiency

Primary Adrenal Insufficiency (PAI)	Secondary or Central Adrenal Insufficiency (SAI)
1.Congenital adrenal hyperplasia (CAH)	**I congenital causes**
2.Bilateral adrenal hemorrhage of the newborn	1. Septo-optic dysplasia (SOD), HESX1, SOX2, SOX3, OTX2
3.Adrenal hemorrhage of acute infection (Waterhouse-Friderichsen syndrome)	2. Pituitary aplasia, pituitary hypoplasia (LHX3, LHX4, OTX2, PROP1)
4.Autoimmune adrenalitis (isolated or part of autoimmune polyglandular syndrome type 1 and 2)	3. Mutations in gene of POMC (Pro-opiomelanocortin)
5.Infection (e.g. tuberculosis, fungal infection, human immunodeficiency virus, cytomegalovirus)	**II acquired SAI**
6.Triple A syndrome or Allgrove syndrome (alacrimia, achalasia, adrenal insufficiency)	1. Trauma
7.Adrenal unresponsiveness to ACTH due to gene mutations	2. Brain tumors
8.Drug effects (mitotane, ketoconazole, aminoglutethimide, metyrapone, megestrol, rifampin)	3. Iatrogenic causes: surgery, cranial irradiation, steroid withdrawal after prolonged administration

is the growth hormone axis. In the acute phase following traumatic brain injury, the hormonal axes most often affected are the gonadotropin axis (41.6 %), the growth hormone axis (20.4 %), the ACTH axis (9.8 %), and the thyroid axis (5.8 %). ACTH production is usually a late function to be lost in pituitary disease and is therefore almost always associated with gonadotropin and GH deficiency. Hormonal dysfunction tends to be at least partially improved in up to 60 % of patients following a traumatic injury [26]. Detection of pituitary antibodies has been used to aid in the diagnosis of post traumatic hypopituitarism [25].

A genetic cause was also considered in our patient but couldn't be confirmed. We did not have possibilities to investigate numerous genes encoding transcriptional factors, identified as critical for pituitary development during early embryonic life. Identified responsible genes for CPHD that includes ACTH deficiency, are numerous: HESX1, LHX3, LHX4, OTX2, PROP1 [10, 27-29]. Complex interplay between congenital and environmental factors with the influence of endogenous factors may result in pituitary malformation. It appears that pituitary talks back to hypothalamus, so that connections between them are vital for normal development of both structures.

To the best of our knowledge, this is the first report of a child with RAP caused by pituitary disorder. We documented the hormonal dysfunction and dysmorphic pituitary. The nature of the SAI in this case, congenital or acquired, remains to be fully elucidated. Secondary adrenal insufficiency usually presents as vague clinical symptoms with the wide range of clinical signs making it difficult to diagnose. Timely recognition and clinical management of adrenal insufficiency are critical to prevent morbidity and mortality.

Conclusion

Adrenal insufficiency of pituitary origin was the cause of RAP in an 8-year-old girl mimicking the abdominal cause of disease. The diagnosis was late and child's suffering was prolonged with possibility of negative outcome. We concluded that the first and more prominent complaint of central adrenal insufficiency in childhood could be RAP, so immediate endocrine evaluation is mandatory. Pituitary insufficiency may evolve over time and children must be kept under careful endocrine follow-up. Untreated hormonal abnormalities could lead to life-threatening AC.

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References

1. Apley J, Naish N. Recurrent abdominal pains: a field survey of 1000 school children. Arch Dis Child 1958; 33:165-170.
2. Kokkonen J, Haapalahti M, Tikkanen S, Karttunene R, Savilahtl E.Gastrointestinal complaints and diagnosis in children: a population basedstudy. Acta Pediatr 2004; 93:880-886.
3. Di Lorenzo C, Youssef NN, Sigurdsson L, Scharff L, Griffiths J, Wald A. Visceral hyperalgesia in children with functional abdominal pain. J Pediatr 2001; 139:838-843.
4. Butler G, Kirk J. Endocrine Emergencies. In: Pediatric Endocrinology and Diabetes. New York: Oxford University Press Ed; 2011. p. 303-328.
5. Growth Hormone Research Society; GH Research Society. Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. J Clin Endocrinol Metab 2000; 85:3990-3993.
6. Fraiser D. Editorial: the treatment of childhood and adolescent growth hormone deficiency-consensus or confusion?J Clin Endocrinol Metab 2000; 85:3988-3989.
7. Gandrud LM, Wilson DM. Is growth hormone stimulation testing in children still appropriate? Growth Horm & IGF Res 2014; 14:185-194.
8. Pampanini V, Pedicelli S, Gubinelli J, Scire G, Cappa M, Boscherini B et al. Brain magnetic resonance imaging as first-line investigation for growth hormone deficiency diagnosis in early childhood. Horm Res Paediatr 2015; 84:323-330.
9. Grimberg A, DiVall SA, Polichronakos C, Allen DB, Cohen IE, Quintos JB et al. Guidelines for growth hormone and insulin-like growth factor-I treatment in children and adolescents: growth hormone deficiency, idiopathic short stature, and primary insulin-like growth factor deficiency. Horm Res Padiatr 2016; 86:361-397.
10. Alatzoglou KS, Webb EA, Tissier Pl, Dattani M. Isolated growth hormone deficiency (GHD) in childhood and adolescence. Endocrine Rev 2014; 35:376-432.
11. Saranac L. The unbearable lightness of prescribing growth hormone. Facta Univ Ser Med Biol 2018; 20(2): 35-39.
12. Sasigarn BA, Rohan H. Pediatric adrenal insufficiency: diagnosis, management and new therapies (Review). International Journal of Pediatrics 2018; (1):1-8. https://doi.org/10.1155/2018/1739831
13. Chaudhuri S. Rao KN, Ommurugan B, Varghese G. Addison's disease mimicking acute pancreatitis. J Clin Diagn Res 2017; 11:12-13
14. Tritos NA. Adrenal Hemorrhage. In: Adrenal Hemorrhage. New York, NY: WebMD. http://emedicine.medscape.com/article/126806. Updated March 3, 2017. Accessed March 13, 2017.
15. Charmandari E, Nicolaides NC, Chrousos GP. Adrenal insufficiency. Lancet. 2014; 383(9935): pp. 2152–2167. doi: 10.1016/s0140-6736(13)61684-0.
16. Nieman LK. Clinical manifestations of adrenal insufficiency in adults. In: Post TW, ed. UpToDate. Waltham, MA: UpToDate. https://www.uptodate.com/contents/clinical-manifestations-of-adrenal-insufficiency-in-adults. Last updated November 4, 2016. Accessed February 19, 2017.
17. Bornstein SR, Allolio B, Arlt W, et al. Diagnosis and Treatment of Primary Adrenal Insufficiency: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2016; 101(2): pp. 364–389. doi: 10.1210/jc.2015-1710.
18. Shulman DI, Palmert MR, Kemp SF. Adrenal insufficiency: still a cause of morbidity and death in childhood. Pediatrics. 2007; 119(2):e484–e494. doi: 10.1542/peds.2006-1612.
19. Turcu AF, Erickson BJ, Lin E, Guadalix S, Schwartz K, Scheithauer BW et al. Pituitary stalk lesions: the Mayo clinic experience. J Clin Endocrinol Metab 2013; 98:1812-1818.
20. Grossman AB. Clinical review: the diagnosis and management of central hypoadrenalism. J Clin Endocrinol Metab. 2010; 95(11):4855-4863. doi: 10.1210/jc.2010-0982.
21. Doknic M, Milijic D, Pekic S, Stojanovic M, Savic D, Manojlovic-Gacic E et al. Single center study of 53 consecutive

patients with pituitary stalk lesions. Pituitary 2018; 21(6):605-614. https://doi.org/10.1007/s11102-018-0914-2
22. Hamilton BE, Salzman KL, Osborn AG. Anatomic and pathologic spectrum of pituitary infundibulum lesions. AJR Am J Roentgenol 2007; 188:W223–W232.
23. Gutch M, Kumar S, Mohd Razi S, Saran S, Kumar Gupta K. Pituitary stalk interruption syndrome: case report of three cases with review of literature. J Pediatr Neurosci 2014; 9:188-191
24. Ruszala A, Wojcik M, Krystynowicz A, Wyrobek L, Starzyk JB. Post-traumatic hypopituitarism caused by pituitary stalk transection. 57 Annual ESPE Meeting 2018, Athens 27-29 Sep: P3-286.
25. De Graff L, De Bellis A, Bellastella A, Hokken Koelega. Antipituitary antibodies in Dutch patients with idiopathic hypopituitarism. Horm Res 2009; 71:22-27.
26. Fernandez-Rodriguez A, Bernabeu I, Isabel Castro A, Kelestimur F, Casaneuva FF. Hypopituitarism folowing traumatic brain injury: determining factors for diagnosis. Front Endocrinol (Lausanne). 2011; 2:25. . doi:10.3389/fendo.2011.00025
27. Kelberman D, Dattani MT. Septo-Optic dysplasia-novel isights into aetiology. Horm Res 2008; 69:257-265
28. Saranac L, Gucev Z. New insights in septo-optic dysplasia. Prilozi 2014; 35:123-128.
29. Saranac L, Bjelakovic B, Djordjevic D, Novak M, Stankovic T. Hypopituitarism occurring in neonatal sepsis. J Pediatr Endocrinol Metab 2012; 25:847-848.

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Case Report

CHRONIC CONSTIPATION IN INFANTS: THINK ABOUT RECTAL DUPLICATION

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Abstract. *Rectum is the least common site of gastrointestinal duplication. Up to now fewer than 100 cases have been reported in the literature. We present two infants with cystic rectal duplications manifested with chronic constipation as a main clinical symptom. The first patient was a 4-year-old boy who was admitted to emergency department because of chronic constipation unresponsive to fiber supplements and laxatives. Digital rectal exam revealed mass adjacent to posterior rectal wall. Abdominal ultrasound and magnetic resonance imaging confirmed oval, homogenous and hypoechogenic cystic mass (87x65x60 mm in size) behind the rectum. The size and location of the cystic mass was confirmed by magnetic resonance imaging. The second patient was an 11-month-old boy who was hospitalized due to rectal bleeding. He was suffering from chronic constipation over the last five months. Digital rectal exam revealed a mass behind the rectum. Abdominal ultrasound and computed tomography showed unilocular cyst (33X33 mm in size) in front of the urinary bladder, partly extending into retrorectal space. Both patients were operated on. Postoperative periods were uneventful in both of them. Cystic rectal duplication must be ruled out in all infants with chronic constipation unresponsive to conservative treatment. Different imaging techniques are currently used to determine the precise size and location of duplication. Surgery is the only possible therapy option.*

Key words: *rectal duplication, children, constipation*

Introduction

Duplications of alimentary tract can occur anywhere from mouth to the anus. The reported incidence of these anomalies is 1 in 4500 [1, 2]. They vary in size, shape (spherical or tubular), and may communicate with the lumen of gastrointestinal tract [3]. Rectum is the least common site of the duplication [4, 5]. There are fewer than 100 cases published in literature [6].

Functional constipation is one the most common reasons parents bring their kids to a doctor. If there is no improvement with medications and dietary changes, organic causes of constipation must be considered. A very rare, but potentially serious cause of constipation in infants is rectal duplication. Delayed diagnosis increases the risk of complications. Therefore, high index of suspicion is needed in all cases of constipation, unresponsive to conservative treatment. In differential diagnosis cystic sacrococcygeal teratoma and anterior meningoceles must be taken into account [7].

Case reports

Case report 1. A 4-year-old boy presented to emergency department for constipation, that was treated with fiber supplements and laxatives over the last six months. Rectal examination revealed cystic mass adjacent to posterior rectal wall; no rectal bleeding was confirmed whatsoever.

Complete blood count and biochemical analyses showed no abnormalities. Initial imaging study included ultrasonographic examination that confirmed extensive, oval, homogenous and hypoechogenic mass (87x65x60 mm) behind the rectum. Magnetic resonance imaging (MRI) confirmed the presence of the cystic mass (Fig. 1). The patient was scheduled for the operative treatment after obtaining written consent from the parents.

We used posterior sagittal approach and revealed the cystic mass presacrally (Figure 2). The mass was completely excised, leaving the small part of the cystic wall in situ, just in the part that shared the wall with the posterior rectum.

The intervention was finished with proper mucosectomy and drainage placing. The postoperative course was uneventful; the drain was removed on the fourth postoperative day. Histopathology exam confirmed duplication cyst with columnar epithelium, mucosal muscularis layer and true rectal mucosa.

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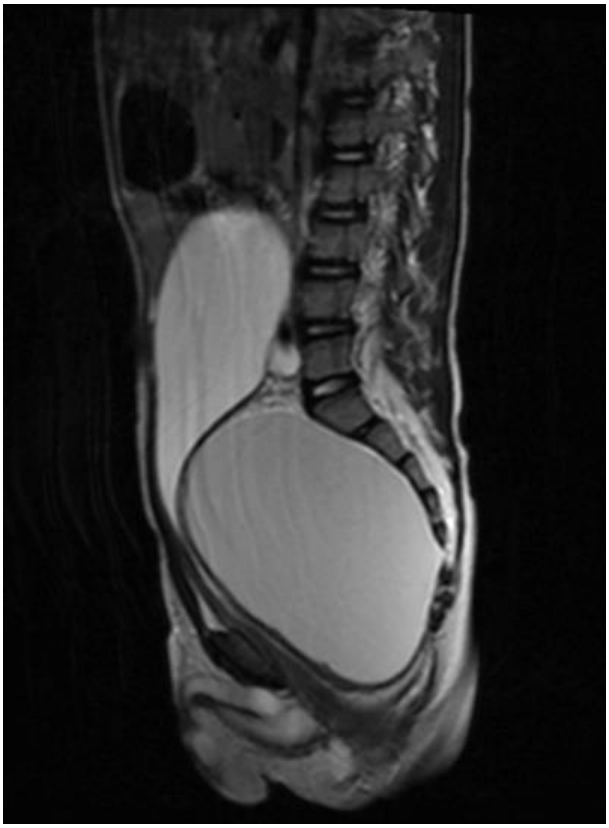

Fig. 1 Large retrorectal cystic mass on sagittal magnetic resonance imaging scan.

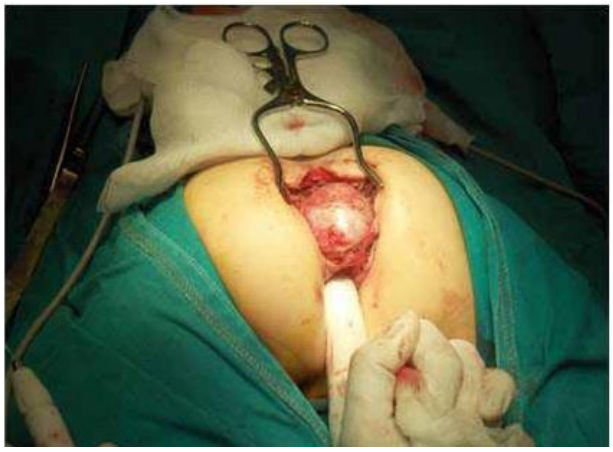

Fig. 2 Operative finding of the retrorectal cystic mass

Case report 2. An 11-month-old male infant was admitted for thorough examination due to rectal bleeding. Patient history revealed chronic constipation over the period of last five months. Digital rectal exam showed cystic mass behind the rectum, that was the most probable cause of the constipation.

Apart from significant anemia (RBC $2.62x10^{12}$/L, Hb – 8.2 g/L), all other laboratory analyses were within the reference values. Computed tomography (CT) scan showed well formed, unilocular cystic formation (33x33 mm in size) in front of the urinary bladder, partly extending into retrorectal space (Figure 3).

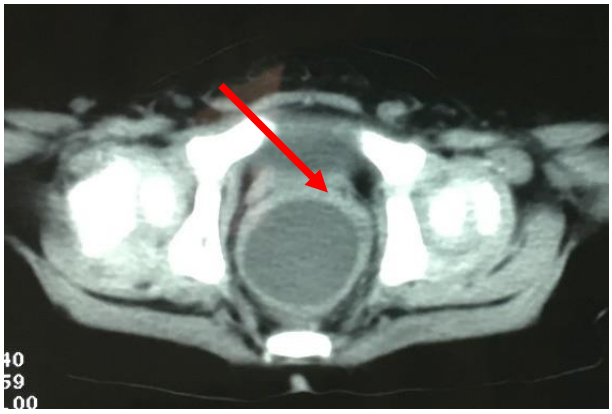

Fig. 3 Rectal duplication on transverse computed tomography scan.

We used posterior sagittal approach for the exposition of the lesion; as no communication with the nearby structures was found, the cyst was been completely enucleated. Having reconstructed the parasagittal muscle complex to preserve the normal sphincter function and continence, the operation was finished with excellent cosmetic result (Figure 4). The postoperative course was uneventful. Histopathology exam proved rectal mucosal lining within the cyst, as well as muscle coat of the wall.

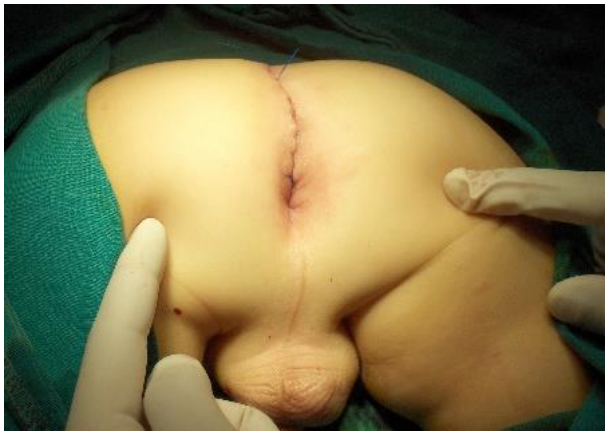

Fig. 4 Excellent cosmetic result after posterior sagittal approach used for the excision of the rectal duplication.

Discussion

Even though duplication anomalies have been known for a long period of time, Ladd was the first to suggest the term duplication in 1937 [8]. They can be found anywhere along alimentary tract (even thoracoabdominaly), causing variety of symptoms depending on their localisation [9]. They vary in size, can be either tubular or spherical, and may communicate with the intestinal tract [1–3].

Although several theories have been proposed, the true etiology of the duplications remains obscure. Persistence of fetal gut diverticula, defects in fetal gut recanalisation, partial twinning and split notochord theory

are some of the many proposed [7, 10]. All of them can be applied to some lesions, yet, no uniform theory has been published so far.

Ladd's criteria for characterising the lesion as duplication are still in use [8]. The lesion has well-developed coat of smooth muscle, inner mucosal membrane resembling any portion of the intestinal tract mucosa, and can have an intimate anatomic association with any part of the digestive tube.

Rectal duplications have bimodal presentation and are mostly seen in perinatal period and during early childhood. They can remain asymptomatic throughout the life span or cause complications (constipation, intussusception, rectal bleeding, sepsis and malignant transformation) [6, 11] or perirectal sepsis [12]. Malignancy is the most serious complication that is rare in childhood [13]. However, it is not so infrequent in adults (7-18% cases) [6].

Even though ultrasonography is widely used as the initial imaging study that offers some information about the presence of the mass itself, precise dimensions and relation to adjacent organs can be obtained only by MRI which is regarded as imaging modality of choice.

Timely diagnosis of rectal duplication is very important in order to prevent wide array of complications. It must be ruled out in all infants with chronic constipation unresponsive to conservative therapy. Rectal bleeding can also be the first sign of duplications. Conservative therapy is largely without results, and the preferred treatment of gastrointestinal duplications is excision [14].

Conclusion

In summary, every child with prolonged constipation, unresponsive to conservative treatment, is to be subjected to sonographic examination, in order to exclude the organic cause of the constipation. The widespread utilisation of ultrasonographic examination helps identify the presence of abdominal and pelvic cystic and tubular lesions and demand further diagnostic imaging modalities.

References

1. Lund DP. Alimentary Tract Duplications. In: Grosfeld JL, O'Neill JA Jr, Fonkalsrud EW, Coran AG, editors. Pediatric Surgery. 6th ed. St. Louis: Mosby; 2006; p 1389-1398.
2. Puligandla PS, Nguyen LT, St-Vil D, Flageole H, Bensoussan AL, Nguyen VH, et al. Gastrointestinal duplications. J Pediatr Surg 2003; 38 740-744.
3. Holcomb III GW, Gheissari A, O'Neill JA Jr, Shorter NA, Bishop HC. Surgical management of alimentary tract duplications. Ann Surg 1989; 209:167-174.
4. Jacquier C, Dobremez E, Piolat C, Dyon JF, Nugues F. Anal canal duplications in infants and children – a series of 6 cases. Eur J Pediatr Surg 2001; 11:1986-1991.
5. Marjanovic Z, Djordjevic I, Slavkovic A, Krstic M. Rectal duplication, rare cause of constipation – case report. Centr Eur J Med 2012; 7(5): 621-623.
6. Harris K, Vellody K. Rectal duplication cyst in a 12 year old female presenting with chronic constipation and rectal bleeding: a case report. Int J Clin Med 2011; 2:5-8.
7. Chandramouli PI, Hossein Mahour G. Duplications of the alimentary tract in infants and children. J Pediatr Surg 1995; 30(9):1267-1270.
8. Ladd WE. Duplications of the alimentary tract. South Med J 1937; 30:363-366.
9. Bhat NA, Agarwala S, Mitra DK, Bhatnagar V. Duplications of the alimentary tract in children. Trop Gastroenterol 2001; 22:33-35.
10. Knight J, Garvin PJ, Lewis E Jr. Gastric duplication presenting as a double esophagus. J Pediatr Surg 1983; 18:300-301.
11. Boleken ME, Kaya M, Ozardali I, Kanmaz T, Yücesan S. Neonatal cecal cystic duplication mimicking intussusception. Pediatr Int 2006; 48:172-173.
12. Flint R, Strang J, Bissett I, Clark M, Neill M, Parry B. Rectal duplication cyst presenting as perianal sepsis: Report of two cases and review of the literature. Dis Colon Rectum 2004; 47:2208-2210.
13. Kuraoka K, Nakayama H, Kagawa T, Ichikawa T, Yasui W. Adenocarcinoma arising from a gastric duplication cyst with invasion to the stomach: A case report with literature review. J Clin Pathol 2004; 57:428-431.
14. Pal K. A treatise on intestinal duplications. Saudi J Med Sci 2015; 3: 8-15.

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