5-Hydroxytriptamine and Skeleton Influence
Clinical study

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5-hydroxytryptamine (5-HT) dually influences skeleton status through positive, indirect central effect and negative, direct, gut-associated impact. Circulating form is usually tested via venous blood sample. A limited number of clinical studies are published on this specific topic. We introduce a cross-sectional study on menopausal women with normal (N=29) and low bone mineral density (N=32) based on lumbar Dual-Energy X-Ray Absorptiometry (DXA) to whom serum serotonin was assessed and found no correlation with bone loss. This aspect confirms conflicting published data regarding the relationship between circulating levels and fracture risk assessment.

Keywords: 5-HT, serotonin, bone, skeleton

5-hydroxytriptamine: a complex clinical panel: 5-hydroxytriptamine (5-HT) or 3-(2-aminoethyl) indol-5-ol, also named serotonin, represents a biogenic amine, traditionally known for its role as neurotransmitter (fig. 1) [1-3]. Exactly the same molecule is found in human body at central level and also outside the blood-vessel barrier but, despite similar biochemical structure, the two locations serve different purposes, which represents a unique profile for a human molecule. The 5-HT synthesis starts with the amino acid L-tryptophan or (2S)-2-amino-3-(1H-indol-3-yl)propanoic acid, and its takes place at both gastrointestinal tract and central nervous system. Peripheral role is autocrine/paracrine/endocrine while centrally it works as neurotransmitter [1-3]. Circulating 5-HT which is clinically tested based on peripheral blood sampling is required for establishing the diagnosis of different pathological conditions like endocrine tumours or carcinoid syndrome [4-6]. Circulating form is mostly derived from platelets storages (platelets do not actually synthesise 5-HT) [1-3]. Human functions of this

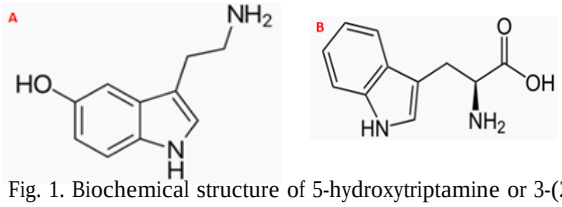

Fig. 1. Biochemical structure of 5-hydroxytriptamine or 3-(2-aminoethyl)indol-5-ol, also named serotonin (A). Its synthesis starts from amino acid L-tryptophan (B)

indoleamine require secondary activation of its specific receptors (5-HT receptors are mainly seven-transmembrane, G-protein-coupled type), for instance: the control of intestinal motility and vascular tone of peripheral blood vessels and brain circulation; the modulation of platelet functionality; and also the regulation of cell growth and metabolism pathways (for instance during embryonic development when it is involved in craniofacial morphogenesis including teeth development) [1,3,7-9]. The clinical conditions described in relationship with malfunction of serotoninergic system are: carcinoid syndrome (excess of serotonin from a neuroendocrine tumour causing diarrhoea, flushes, bronchospasm, etc.), irritable bowel syndrome; psychiatric and neurological diseases as migraine, depression, mood disturbances, multiple sclerosis; pulmonary hypertension; abnormal modulation of immune response, etc. [10-14].

Serotonin and bone: One of the modern topics regarding serotonin signal transduction is the regulation of bone status from a dual point of view: central neurotransmitter is an indirect contributor to bone mass enhancement while peripheral 5-HT causes bone loss, probably through direct local actions. Central 5-HT has a positive effect on bone mass, an indirect one since it never actually gets to the skeleton (it does not cross the brain-vessels barrier). Peripheral 5-HT is produced by gut cells and is transported through blood at platelets levels (this form is actually the only one accessible for clinicians by routine venous blood sampling). By this route 5-HT arrives to bone and expresses direct negative effects on bone mass. Also, 5-HT may be locally produced at the skeleton level where it displays autocrine/paracrine actions (fig. 2) [15,16]. The dynamic link with skeletal field has yet unanswered questions. The place of routine serotonin assay from venous sample to assess the bone deterioration is still incompletely known since 5-HT influence on bone may be a cocktail of divergent actions [17].

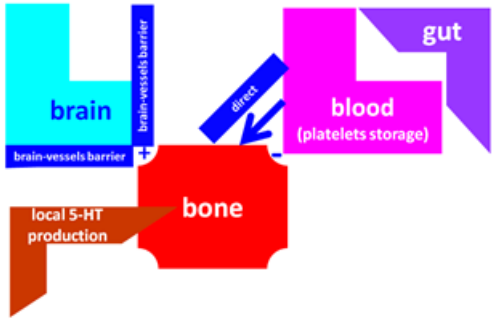

Fig. 2. 5-hydroxytriptamine (5-HT) and potential bone influence

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We aim to introduce a clinical study on menopausal women without prior bone conditions or other diseases/ drugs that might influence 5-HT to assay the blood 5-HT in relationship with loss of bone mineral density (BMD) as reflected by central Dual-Energy X-Ray Absorptiometry (DXA) and to connect these clinical observations to prior published data on the same topic.

Experimental part

Method

This is a cross-sectional observational study on a tertiary centre of endocrinology from Romania. The study was conducted between 2016 and 2017.

The collected data are based on anamnesis (medical history and records), clinical evaluation (body mass index or BMI), blood assays (fasting 5-HT via venous sampling, results provided by high-pressure liquid chromatography assay), central lumbar DXA (GE Lunar Prodigy device), and FRAX-calculated risk of fracture (table 1). DXA provided Bone Mineral Density (BMD) and associated T-score [18]

FRAX algorithm was used for Romania, and did not include bone mineral density, neither trabecular bone score for estimation [17] 10-year absolute risk of fracture was appreciated for major osteoporotic fractures meaning clinical spine, forearm, hip, shoulder fractures (RISK1), and also for hip fracture (RISK 2) using the online free download model for specific country [19]. Statistical analysis: database was created in Excel and exported in SPSS 21; level of significance was considered at $p<0.0.5$.

The patients met the inclusion and exclusion criteria introduced in table 2. The research was performed by including the subjects into two groups based on lumbar DXA – BMD and associated T-score, as directly calculated by DXA device software: GROUP 1 with normal T-score (± 1 SD) (SD = standard deviation) and GROUP 2 with abnormal T-score based on BMD loss according to central DXA (< - 1 SD).

Results and discussions

A total of 61 subjects were included: N=29 in GROUP 1, respective N=32 in GROUP 2.

The baseline parameters provided by medical history showed similar age, age at menopause, and years since menopause between the groups and a statistical significant

Parameter	Method of collection
Prevalent fragility (osteoporotic) fracture	▪ Anamnesis ▪ Medical records ▪ Profile X-Ray of the spine at thorax and lumbar level
Body Mass Index (BMI)	Calculation based on formula = (weight) X (height)^2
Serotonin (5-HT)	Venous blood collection (fasting state; high-pressure liquid chromatography)
Bone Mineral Density (BMD)	Lumbar L1-4 Dual-Energy X-Ray Absorptiometry (DXA) based on a GE Lunar Prodigy device
T-score (SD)	Based on BMD (WHO criteria); directly provided by DXA device
10-year absolute risk of fracture for major osteoporotic fractures like clinical spine, forearm, hip, shoulder fractures (RISK1), and for hip fracture (RISK 2)	FRAX online calculation tool for Romania

Table 1
THE PARAMETERS ANALYSED IN CLINICAL STUDY WHICH ENROLLED MENOPAUSAL WOMEN

Inclusion criteria	Exclusion criteria
adult patient	neuroendocrine tumours carcinoid syndrome
menopausal status	known active cancers of any origin
age of 50 years or older	current or prior anti-osteoporotic drugs exposure
informed consent of the patient	current or prior estrogens therapy
lumbar DXA scan with technically achievable data	current or prior anti-depressant medication
	psychiatric disorders
	neurological conditions
	current anticoagulant drugs, glucocorticoid exposure
	bone metabolic conditions (like Paget's disease, primary hyperparathyroidism)

Table 2
THE INCLUSION AND EXCLUSION CRITERIA IN CLINICAL TRANSVERSAL NON-INTERVENTIONAL STUDY

Parameter	Group	mean	SD	units	p value
Age	GROUP 1	59.13	7.65	years	0.24
	GROUP 2	61.31	6.89		
Age of menopause	GROUP 1	46.78	5.06	years	0.35
	GROUP 2	48.00	4.95		
Years since menopause	GROUP 1	12.60	9.29	years	0.75
	GROUP 2	13.34	0.09		
Body Mass Index	GROUP 1	29.70	6.24	kg/sqm	0.02
	GROUP 2	26.45	5.02		

Table 3
THE BASELINE FEATURES OF STUDIED GROUPS

Parameter	Group (number)	Number (N/%)
Smoking	GROUP 1 (29)	7/24.13
	GROUP 2 (32)	6/18.75
Prevalent fragility fractures	GROUP 1 (29)	2/6.89
	GROUP 2 (32)	4/12.50

Table 4
THE DISTRIBUTION OF PATIENTS WHO ARE CURRENTLY SMOKERS AND HAVE PREVALENT OSTEOPOROTIC FRACTURES

difference between BMI, a parameter that were further adjusted for statistical analyze (table 3). The percent of current smokers and prevalent fragility fractures (as used by FRAX algorithm) is displayed in table 4. DXA - BMD results at lumbar spine confirmed the statistical significant difference between the two groups (table 5). 10-year absolute risk of fracture was similar between the groups (table 6). The serotonin values showed that GROUP 1 had a value of 168.09 ± 76.37 ng/mL (normal levels between 80 and 200 ng/mL), and GROUP 2 had 192.73 ± 98.74 ng/mL. No statistical significant difference between the groups with normal or damaged DXA - BMD was found regarding circulating 5-HT ($p=0.28$). A percent of 6.89% (N=2), respective of 6.25% (N=2) in each studied group, had 5-HT lower than normal limits. BMD and RISK 1, respective RISK 2 in these subjects were not different from patients with normal blood 5-HT ($p>0.05$).

Based on inclusion and exclusion criteria, blood serotonin was slightly higher than upper normal limit only in 2 subjects from GROUP 2 (representing 6.25% from the group). These females had not different BMD, RISK 1, and RISK 2 from the population of GROUP 2 with normal serum 5-HT ($p>0.05$).

Linear regression (± BMI adjustment) between 5-HT and BMI, BMD, respective RISK 1, and RISK 2 did not reach statistical significance for neither group ($p>0.05$).

Only a few clinical studies have been published until now regarding 5-HT assessment on different populations at high risk for bone mass deterioration and consecutive increase of fracture risk.

We selected menopausal women with low BMD to highlight potential associated changes of blood 5-HT. Based on our observations, the use of 5-HT assays did not reflect the differences of bone anomalies as they are included in DXA-BMD loss or increased fracture probability as projected by RISK 1 and 2. The simple blood test of serotonin might not be enough to highlight its complex influence on skeleton status. Priory published clinical study on menopausal women (but with less restrictive inclusion/exclusion criteria) found that 5-HT has an insignificant correlation with bone turnover markers [17]. As collateral observations we mention the fact that the subjects had relatively low fracture risk for 10 years according to FRAX model. As mentioned by inclusion criteria, the patients with current glucocorticoid exposure were not included to avoid the cortisone influence on bone metabolism and,

Parameter	Group (N)	mean	SD	units	p value
Lumbar L1-4 BMD-DXA	GROUP 1 (29)	1.161	0.252	g/sqcm	<0.0005
	GROUP 2 (32)	0.934	0.094		
Lumbar L1-4 T-score	GROUP 1 (29)	1.17	0.14		<0.0005
	GROUP 2 (32)	-1.9	0.74		

Table 5
DXA (DUAL-ENERGY X-RAY ABSORPTIOMETRY) AT LUMBAR SPINE LEVEL:
BMD (BONE MINERAL DENSITY) AND T-SCORE BETWEEN THE TWO GROUPS

Parameter	Group (N)	mean		SD	units	p value
RISCK 1	GROUP 1 (29)	3.58		1.74	%	0.12
	GROUP 2 (32)	5.06		4.82		
RISCK 2	GROUP 1 (29)	0.95		0.79	%	0.17
	GROUP 2 (32)	2.55		1.48		

Table 6
10-YEAR PROBABILITY OF MAJOR OSTEOPOROTIC FRACTURES (RISK 1) AND HIP FRACTURE (RISK 2) BASED ON FRAX ALGORITHM

potentially, its influence on 5-HT levels. Moreover, most of the enrolled population did not have prevalent fractures to influence the 10-year probability of fracture. We choose extremely restrictive criteria of patients' selection to exclude any potential clinical influence on serotoninergic system and 5-HT assays.

As limits of the study we mention small sample size, the effect of surgical or spontaneous menopause was not studied. Higher BMI in group with normal T-score required adjustment for 5-HT analysis.

5-HT represents an important molecule in human body, including the bone perspective. 5-HT is found mainly in the gut (95%) and only 5% in brain [20]. The intestinal production serves for local gut purposes but it is up taken by blood at the level of circulating platelets where it is used during clotting and vasoconstriction processes [20]. By this path, 5-HT is delivered to bone where it directly acts opposite to central 5-HT which never get directly to skeleton even indirectly clearly influences its metabolism [20,21]. 5-HT of gut origin decreases bone formation by inhibiting osteoblast proliferation via its receptors on pre-osteoblasts [20,21].

Modern data suggests that bone cells like osteoblasts, osteoclasts, and osteocytes may even produce 5-HT and express SERT (serotonin-reuptake transporter), and also they all express serotonin specific receptors [21,22]. Ongoing studies are designed to introduce new drugs that target bone-related serotoninegic pathways [23]. For instance, serotonin 6 G-protein-coupled receptor (or 5-HT6R), which is typically used for neurological conditions, was found with a higher expression in bone, especially related to osteoblasts activity [23]. Murine experiments with ST1936, a 5-HT6R activator, blocks bone regeneration while SB258585 inhibits 5-HT6R and it might represent a future therapeutic option for bone repair [23]. Local serotonin is used for skeleton daily metabolism, especially remodelling processes, based on autocrine and paracrine actions (also named autacoid) [21,22] (fig. 2). Intestinal serotonin production is modulated by gut microbiota and this represents one of the most recent theories of food - bone regulation via 5-HT [24,25]. The exact pathways of intestinal microbiome to modulate skeleton biology is still a matter of debate [24-26]. Enterochromaffin cells-produced serotonin also influences glucose and lipid profile as does for the skeleton being a strong player of the bone-fat crosstalk [27]. Rat experiments proved a reduction of osteoblasts-derived bone turnover markers like alkaline phosphatase and osteocalcin as effect of 5-HT which may be a part of local skeletal microenvironment with negative impact on bone mass [28]. However, when translate these data to human studies a less clear relationship was detected.

Central 5-HT inhibits the sympathetic pathways of bone forming inhibition through actions on hypothalamus, thus expressing a final indirect positive effect on bone mass and skeleton growth (fig. 2) [21]. The central synthesis of 5-HT starts from tryptophan (as seen in gut enterochromaffin system), an essential amino acid, which is then converted to 5-hydroxytryptophan by the enzyme called tryptophan hydroxylase (a reaction which is a biopterin-dependent monooxygenation and it is a rate-limiting step regarding 5-HT production) [29-31]. After this step, 5-hydroxytryptophan becomes the substrate of enzyme aromatic amino acid decarboxylase, generating serotonin (through a decarboxylation reaction) [29-31]. Until 1988, tryptophan hydroxylase type 1 was considered responsible for all serotonin synthesis (its gene is located on chromosome 11), but in 2003 was confirmed that actually brain 5-HT is specifically provided by tryptophane hydroylase type 2 (its gene is located on the long arm of chromosome 12) [32,33]. Brain tryptophan also serves as a substrate for other molecules, for instance, melatonin (or N-acetyl-5-methoxy tryptamine), tryptamine, etc. [29-31]. The same bone effect as 5-HT is consistent with pineal gland - derived melatonin, expressing indirect bone mass stimulator effect [29-31]. It seems that tryptophan metabolites are key elements in mechanisms of bone metabolism under physiological and pathological circumstances [34]. Generally, the hypothalamus offers a central control of skeletal regulatory system, a part from its well-known roles in water, energy, and food behaviours [35]. Since brain is a dynamic and complex regulator of bone activity, potentially a dual inter-connection is actually active since bone might also influence the brain through molecules as osteocalcin and serotoninergic central-peripheral system [36]. Another complex chapter that links brain to bone via serotonin is depression - related bone loss as well as negative skeleton effects of anti-depressant medication like selective serotonine reuptake inhibitors [37-39]. For this reason, the current study excluded patients with depression or anti-depressant exposure not to influence the 5-HT status.

Out study confirms other similar clinical studies on circulating serotonin. We selected the most recent clinical studies aiming to identify a potential use of 5-HT assay in daily clinical practice regarding conditions with associated bone mass damage without exposure to psychiatric medication which specifically interfere with serotoninergic pathways like selective serotonin reuptake inhibitors. A study published on 2017 on 205 patients with rheumatoid arthritis, a condition with high bone loss and potentially linked with increased blood 5-HT, found an inverse week correlation between serum serotonin levels and BMI ($r= -0.218$, $p=0.005$), respective osteoprotegerin in women ($r= -0.26$, $p=0.022$); a positive correlation with bone resorption marker CTX in antiosteoporotics naïve men ($r= 0.59$, $p<0.001$), respective sclerotin at the same population group ($r= -0.374$, $p<0.05$) [40]. 5-HT was identified as potential BMD predictor in some groups but without a general pattern to use in daily practice (like the results of our study) [40]. A large study on 121 premenopausal subjects, 124 postmenopausal women and 168 men also used serum 5-HT (competitive enzyme-linked immuno-sorbent assay) to correlate with bone damage as reflected by DXA. In premenopausal group, 5-HT was inversely associated with unadjusted lumbar BMD ($r= -0.23$, $p<0.05$) but not after weight adjustment (neither in men group). In postmenopausal group, some bone traits as whole body BMD positively correlated with serotonin, opposite to our results, also on menopausal subjects [41]. In one study from 2018, the assessment of serum serotonin was done on 202 adult males who were followed for 3.7 years and it was negatively weak correlated with lumbar BMD ($r= -0.174$, $p=0.028$) at start and then the statistical significance was lost during follow-up; nevertheless baseline 5-HT did not predict the rate of annual bone loss, neither discriminate the patients with prevalent fractures from subjects without [42]. As mentioned before, similar transversal data we obtained on menopausal women. Bone loss in young females with anorexia nervosa was analysed from bone turnover point of view, including 5-HT, in one study published in 2016. Serum serotonin was statistically significant lower than control, and directly associated with BMI, leptin and inversely correlated with bone resorption marker CTX [43]. Another study on 117 premenopausal Chinese women and 262 postmenopausal

subjects revealed the relationship between 5-HT and different parameters and the conclusions were: pre- versus post-menopausal status associates higher 5-HT; in post-, not pre-menopausal women 5-HT correlates with lumbar and femoral neck BMD when adjusted for age and BMI [44]. This particular type of correlation was not sustained by our similar cohort and by many of already published studies as priory mentioned.

Conclusions

5-hydroxytryptamine influences skeleton status through dual actions: positive, indirect central effect and negative, direct, gut-associated impact. Another contributor is locally generated monoamine playing an autocrine role. A limited number of clinical studies are published on this specific topic, as our observational cohort in menopausal women with normal and low bone mineral density, confirming the conflicting results of the relationship between serum 5-hydroxytryptamine concentrations and fracture risk as provided by central DXA.

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