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Runt-Related Transcription Factor 2 (Runx2) Measurement in Phytoestrogen-Induced Bone: A Comparison of Western Blot and Immunohistochemistry Methods

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Estrogen deficiency can contribute to osteoporosis in postmenopausal women. Phytoestrogens are becoming more widely recognized as potential estrogen replacement therapy. The administration of phytoestrogens can cause bone formation, which is marked by an increase in Runx2 expression in osteoblast cells and can be seen using western blot and immunohistochemistry approaches. This review aimed to compare the detection methods of Runx2 in phytoestrogen-induced bone tissue using western blots and immunohistochemistry. Selectivity, sensitivity, processing time, and cost-effectiveness were the parameters that were compared. This review was done by identifying articles in several databases (Google Scholar, PubMed, and Science Direct). The process of selecting the articles used the PRISMA guidelines to create a flowchart with inclusion and exclusion study criteria. Meta-synthesis was done to analyze, identify, and interpret all of the data in the articles systematically. 70 articles in total were obtained from the selection process, with 21 articles being relevant to the topic. The result shows that the selectivity and sensitivity of western blot for detecting Runx2 on tissue were 93.5–100%, respectively, whereas immunohistochemistry selectivity and sensitivity were 45–99.5%, respectively. Compared to immunohistochemistry, western blot can save up to 57.26%. Immunohistochemistry takes 46 hours to process, while Western blot takes 25 hours and 20 minutes. In comparison to immunohistochemistry, the western blot is more selective, sensitive, rapid and affordable for detecting Runx2 in bone tissue.

Keywords: Immunohistochemistry; Phytoestrogens; Runx2; Western Blot.

Estrogen deficiency or decreased estrogen production in postmenopausal women is common, and it can occur spontaneously as women get older[1]. Estrogen deficiency can enhance the activity of osteoclasts, resulting in increased bone resorption, as well as blocking the development of osteoblast precursors, preventing bone growth. Osteoporosis can be caused by an imbalance in the bone remodeling process[2, 3]. Osteoporosis is defined by a reduction in bone mineral density (BMD) and

damage to the microarchitecture of bone tissue, both of which increase the risk of fracture[4, 5].

According to statistics from the International Osteoporosis Foundation, more than 200 million people worldwide suffered from osteoporosis in 2016[6]. According to Amelia's research[7], the prevalence of osteoporosis in Indonesia is 18-36% in women and 20-27% in males under the age of 70. Women account for 53.6% of those above the age of 70, while males account for 38%. Furthermore, one in every four Indonesian women between the ages of 50 and 80 is at danger of getting osteoporosis. In Indonesia, women are four times more likely than males to develop osteoporosis. The prevalence of osteoporosis in women increases with age, according to these findings. It's possible that this is linked to estrogen deficiency in postmenopausal women[8].

Hormone replacement therapy (HRT) can be used to replace estrogen loss in postmenopausal women. Long-term use of HRT, on the other hand, has the potential to be carcinogenic (an increase in endometrial cancer and breast cancer), embolic, and even cause stroke, all of which can result in mortality[9-11]. The results of research into the use of natural components to replace the function of estrogen with minimal negative effects have been published, and the findings will lead to the usage of phytoestrogens[12].

Phytoestrogens are described as compounds with a structure similar to 17-estradiol or compounds that have a high affinity for the estrogen receptor and can have a therapeutic effect similar to estrogen[13-16]. Phytoestrogens have been shown to increase osteoblastic activity while inhibiting the development of osteoclasts[17]. The phytoestrogens in the n-hexane extract and the fraction resulting from the separation of Marsilea crenata leaves could promote the differentiation of MC3T3-E1 osteoblast cells, according to Ma'arif et al[18]. Daidzein, a phytoestrogen, can boost OCT1 osteoblast cell proliferation and differentiation[19]. The phytoestrogen puerarin can promote osteogenesis in MC3T3-E1 osteoblast cells, according to Wang et al[20]. An increase in runt-related transcription factor 2 (Runx2), a transcription factor involved in osteoblast cell development, can be linked to increased bone production by phytoestrogens[21].

Western blot (WB) and immunohistochemistry (IHC) approaches can be used to examine Runx2 bone tissue. Both of these approaches use antigen-antibody interactions to detect proteins[22-24]. The advantages and disadvantages of immunohistochemistry and western blot procedures can be seen based on numerous parameters such as selectivity, sensitivity, processing time, and cost-efficiency. These four factors must be taken into account when choosing protein observation methods. A method must be selective in order to be able to distinguish the target protein among other components in the sample carefully and thoroughly[25]. The technology adopted must also be sensitive enough to detect protein at very low concentrations[26]. The time and expense of developing a method must also be taken into account in order to develop a method that is both effective and efficient. So, it is intended that this comparative literature analysis will provide information on the best approach to observe Runx2 depending on the parameters that have been specified.

MATERIALS AND METHODS

Materials

Criteria of collecting data

Inclusion criteria used to select the articles were: (i) Studies from original articles that referred to the effects of phytoestrogens on bone formation; (ii) Studies that measured Runx2 using immunohistochemistry or western blot; (iii) Publication from 2010 until 2021; (iv) Publication using English and Indonesian language; and (v) Articles that are full text or can be fully accessed.

The exclusion criteria applied to each article were: (i) Original article that published before 2010, (ii) Article in another language besides English and Indonesian; and (iii) article that was not in full text or could not be fully accessed.

Collecting strategy and article selection

Articles were searched using PubMed, Google Scholar, and Science Direct databases with phytoestrogens, western blot, immunohistochemistry, runx2, bone, tissue as the keywords. Articles were checked for the suitability of their titles and abstracts with the research topic. In addition, it was also checked for the possibility of duplication of articles among the databases

used. Then the feasibility test was carried out by reading the entire text in the selected articles based on predetermined criteria. In addition, the selected articles are full text and open access script with the theme of the study related to the effect of phytoestrogens on the expression of Runx2 in bone tissue by using immunohistochemistry or western blot methods. Relevant articles are taken as primary data[27, 28].

Methods

The method for searching and selecting research articles is illustrated using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline [27, 29, 30]. Based on the PRISMA guideline with database and keywords, 70 articles were obtained, but only 21 articles had relevant topics, all of which were published within the last 10 years (Figure 1). From this quality assessment, 12 articles were obtained from Google Scholar, 3 articles from PubMed, and 6 articles from Science Direct (Figure 2).

Data Analysis

Articles data obtained from the PRISMA diagram were then analyzed qualitatively. Qualitative data analysis is used to describe and compare the results of research articles found. 21 Articles were used to compare immunohistochemical and western blot methods through Runx2 expression. This comparison covers aspects of numerous parameters such as selectivity, sensitivity, processing time, and cost-efficiency.

RESULT AND DISCUSSION

In Vivo Study of Phytoestrogen Effects on Bones

Bone tissue is composed of three main cells, namely osteoblasts, osteoclasts, and osteocytes. The cells that have a major role in bone formation are osteoblasts. These cells differentiate from mesenchymal stem cells, mediating bone formation[32]. Osteoblast differentiation is due to the commitment of osteogenic precursors from mesenchymal stem cells, followed by differentiation into pre-osteoblasts, immature osteoblasts, and mature osteoblasts[33]. Runx2, which is a transcription factor for osteoblast differentiation, is expressed by mesenchymal stem cells during early embryonic development and acts as a major regulator of cells leading to osteoblasts[34]. Thus, Runx2 is considered as one of the earliest and most specific markers during the process of osteogenesis[35].

Runx2 observations were made on ovariectomized rats and mice[36-38]. Osteoporosis in either ovariectomized rats or mice showned many pathophysiological features similar to postmenopausal osteoporosis in humans, so either ovariectomized rats or mice can be good

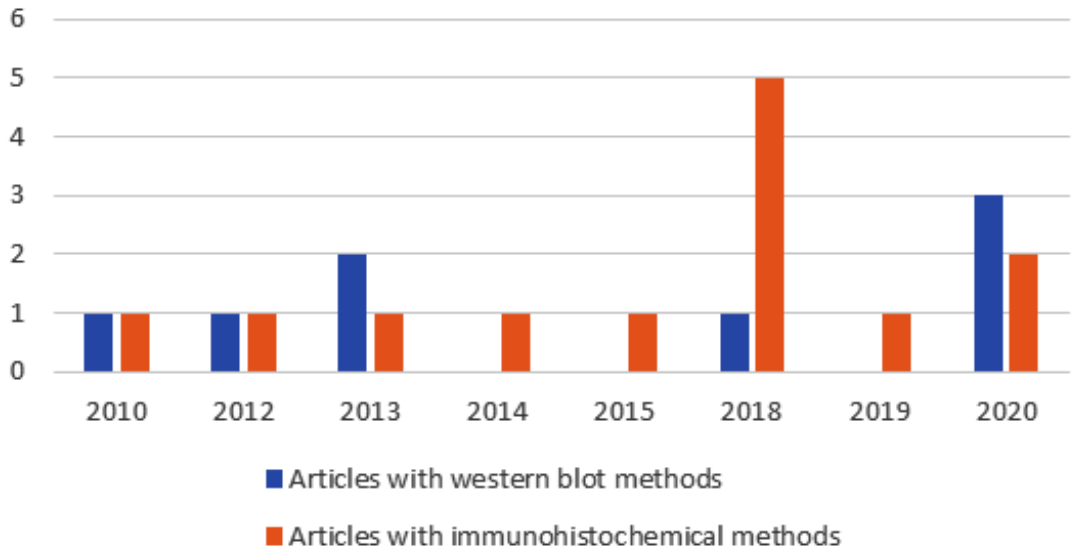

Fig. 1. Years publication of research articles

osteoporosis models[38]. Ovariectomy induces estrogen deficiency in experimental animals, which results in osteoporosis. Experimental animals that were ovariectomized were shown to have decreased expression of Runx2, which resulted in decreased bone formation[36, 39].

Experimental animals with estrogen deficiency were given phytoestrogen therapy. Then, the effect of these phytoestrogens on bone formation was analyzed by measuring the expression of Runx2. Phytoestrogen compounds were given in various doses and intervals of administration, which can be seen in Table 1. In this study, positive control (estrogen) and negative control (untreated) controls were also used as comparisons. The effect of giving phytoestrogens on Runx2 expression can also be seen in Table 1.

Based on Table 1, it can be seen that phytoestrogens can increase the expression of Runx2 in the bone tissue of experimental animals. Runx2 is a key factor required for osteogenic differentiation and bone development of mesenchymal stem cells[40]. Increased expression of Runx2 stimulates mesenchymal cells to differentiate into osteoblasts[41]. So that the increase in Runx2 expression is also an indication of an increase in bone formation.

Research by Liu et al[36] showed that administration of glabrene from the roots of *Glycyrrhiza glabra* (licorice) to ovariectomized rats could significantly increase Runx2 expression compared to negative controls ($p<0.05$). According to research by Zhang et al[38], resveratrol is a natural polyphenolic compound with a structure similar to the estrogen diethylstilbestrol and is found in grapes, peanuts, canapes, and so on. Resveratrol can competitively bind to the estrogen receptor and exert an estrogen-like effect. Administration

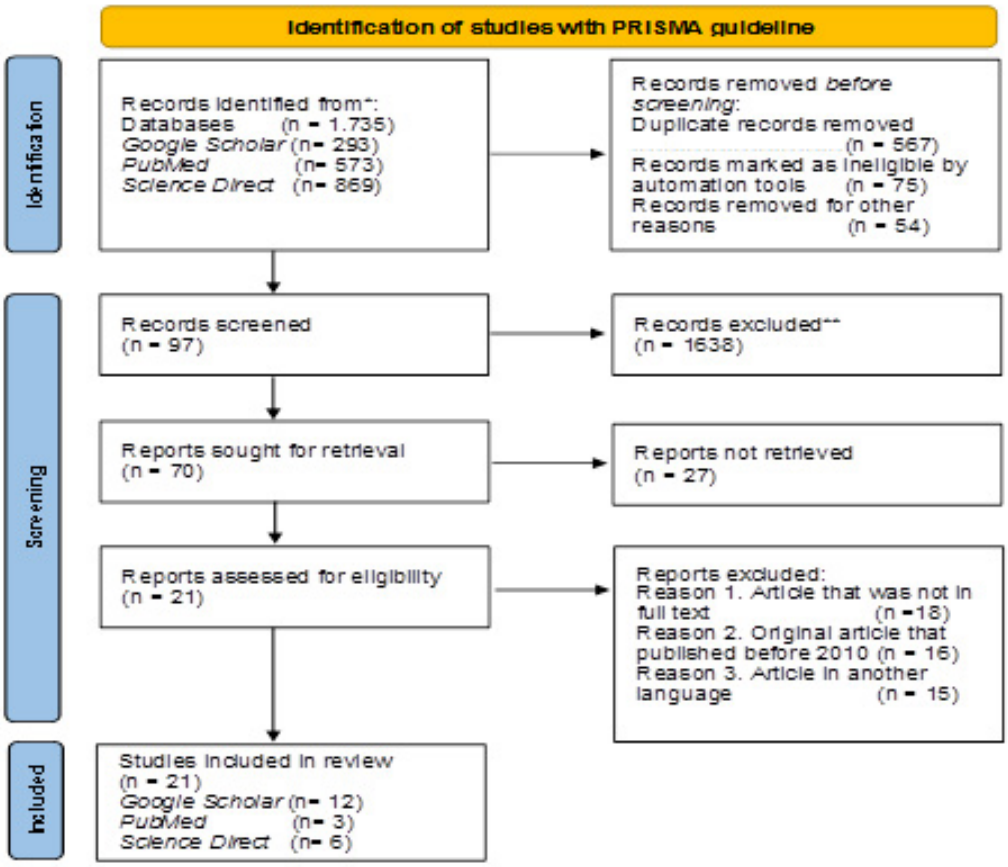

Fig. 2. Flow diagram of the study selection process following PRISMA guideline[31]

Table 1. Effects of Phytoestrogens on Runx2 Expression

No.	Phyto-estrogen	Methods	Doses	Administration Interval	Increased Runx2 Activity	Signs of Bone Formation	Citation
1.	Glabrene	WB	25; 50; and 100 mg/kg	13 weeks	✓	Increased expression of Runx2, catenin, Lrp 5, Osx, OPG and BGP	36
2.	Phenolic Acid	WB	100 g	14-40 days	✓	Increased expression of ALP, osteocalcin, and Runx2	47
3.	Resveratrol	WB	50; 100; and 200 mg/day	12 weeks	✓	Decreased expression of NF-êB and increased expression of Smad7, BMP-2, and Runx2.	38
4.	Icariin	WB	600 mg/kg	12 weeks	✓	Increased expression of Runx2 and OPG.	42
5.	Genistein	WB	250mg/kg	8 weeks	✓	Increased expression of Runx2 and osteocalcin.	50
6.	â-Ecdysterone	WB	5 or 10 mg/kg	5 weeks	✓	Increased expression of Runx2 and BMP-2	51
7.	Isoflavonescontained in soy protein isolate (SPI)	WB	-	2 weeks	✓	Decreased expression of caveolin-1, and Increased expression of BMP-2 sitosol, Smad and Runx2	52
8.	Isoflavones contained in *soy protein isolate* (SPI)	WB	-	2 weeks	✓	Increased expression of Osterix, Runx2 and â-catenin, also decreased expression of caveolin-1	53
9.	Emodin	IHC	100 mg/kg	3 months	✓	Increased expression of Runx2, osterix, type 1 collagen, osteocalcin, and ALP	41
10.	Prunetin	IHC	0,125 mg/kg and 0, 25 mg/kg	2 weeks	✓	Increased expression of Runx2	54
11.	Total ûavonoids of Rhizoma drynariae (TFRD)	IHC	20 mg/kg	18 days	✓	Increased expression of Runx2 and BMP-2	40
12.	Cinnamaldehyde	IHC	25 mg/kg; 50 mg/kg; 75 mg/kg	12 weeks	✓	Increased amount of osteoblas, Increased expression of ALP, Runx2, osteocalcin and type IQ1 collagen. also decreased amount of osteoklas	55
13.	Isoformonetin	IHC	1; 10; and 25 mg/kg	12 weeks	✓	Increased expression of type 1 collagen, osteokalsin and Runx-2	56
14.	Kaempferol	IHC	5.0 mg/kg	6 days	✓	Increased of Runx2 through Wnt signaling pathway	49
15.	Icaritin	IHC	8 mg	2 weeks	✓	Increased expression of Osterix and Runx2	57
16.	Notoginsenoside R1	IHC	100 iL	4 weeks	✓	Increased expression of Runx2 and osteocalcin	58
17.	Cinnamaldehyde	IHC	50 mg/kg	5 weeks	✓	Increased expression of Runx2 and ALP	59
18.	Ginsenoside Rg1 (G-Rg1)	IHC	0,1; 1; 10; 50; 100 ug/mL	4, 8, and 12 weeks	✓	Increased expression of Runx2, osteocalcin, RANKL and OPG	32
19.	Kaempferol	IHC	5mM	12 days	✓	Increased expression of Runx2 and osterix	60
20.	Icariin	IHC	25 mg/ kg	12 weeks	✓	Increased expression of Runx2 and osterix	37
21.	Icariin	IHC	20mg/kg	2 weeks and 3 months	✓	Increased expression of type 1 collagen, Runx2 and osteocalcin	39

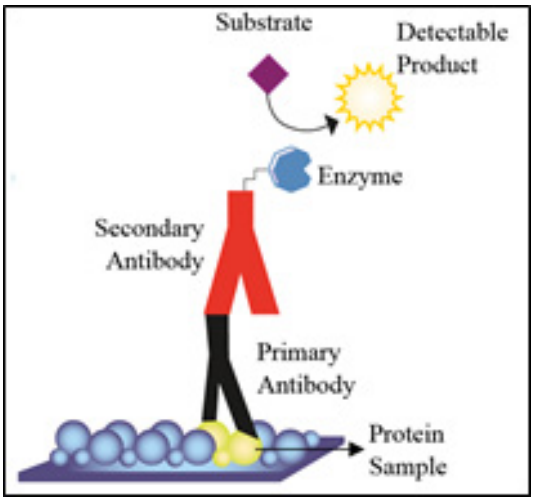

Fig. 3. Indirect method on western blot [68]

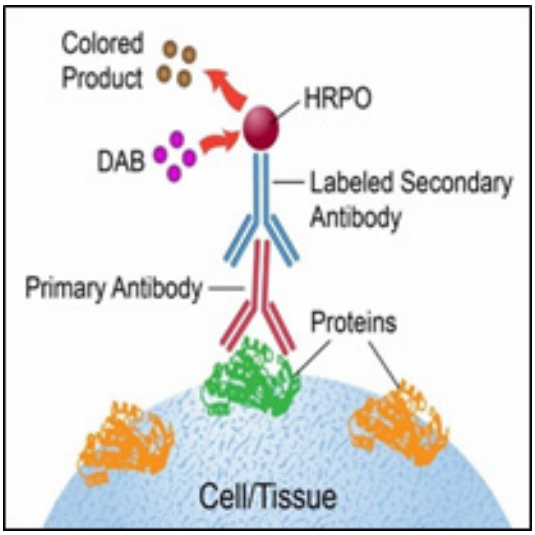

Fig. 4. Indirect method on immunohistochemistry[73]

Table 2. Processing time of western blot

No.	Step	Time required	Citation
1.	Sample preparation	2 hours 25 minutes	36, 38, 91, 92
2.	Protein quantification	2 hours	93
3.	Elektrophoresis	2 hours	77, 94
4.	Transfer of proteins from gel to membrane	1 hours 40 minutes	77
5.	Immunodetection		
	Blocking (room temperature)	2 hours	36, 38
	Primary antibody incubation (on 4°C)	12 hours	36, 38
	Washing	45 minutes	38
	Secondary antibody incubation (room temperature)	2 hours	38
	Total	25 hours 20 minutes	

Table 3. Processing time immunohistochemistry

No.	Step	Time required	Citation
1.	Sample preparation		
	Fixation	24 hours	95
	Planting and cutting	6 hours 15 minutes	71, 72
	Deparaffinization	1 hour 5 minutes	71
2.	Antigen retrieval	20 minutes	41
3.	Blocking (room temperature)	30 minutes	95
4.	Primary antibody incubation (at 4°C)	12 hours	32, 37, 39, 56
5.	Washing	15 minutes	96
6.	Secondary antibody incubation (room temperature)	1 hour	54, 56
7.	Washing	15 minutes	95
8.	Addition of chromogen substrate	5 minutes	95
9.	Washing	15 minutes	95
	Total	46 hours	

of resveratrol to ovariectomized rats could significantly increase Runx2 expression compared to negative control ($p<0.05$). According to research by Zhang et al[42], administration of icariin, which is the active ingredient of Herba Epimedii in ovariectomized rats, can significantly increase Runx2 expression compared to negative control ($p<0.05$). The results of immunohistochemical analysis also showed that administration of icariin at a dose of 25 mg/kg in ovariectomized rats could significantly increase Runx2 expression compared to negative controls ($p<0.01$)[37].

All tested compounds on experimental animals with estrogen deficiency in Table 1 can replace the function of estrogen in binding to the estrogen receptor, although they have different structures. The relationship between chemical structure and biological activity can be done by associating certain functional groups with certain biological responses. Compounds with the same functional group will have the same activity[43]. In this study, examples of isoflavonoid compounds are glabrene, genistein, daidzein, equol, isoformonetin, and prunetin. Isoflavones are phytoestrogen compounds because they have a chemical structure similar to the hormone estrogen, namely 17â-estradiol. Isoflavone compounds are able to bind to estrogen receptors then provide physiological activity as an estrogen hormone[44]. The most important chemical group of isoflavones is the phenolic ring, which is a binding site on the estrogen receptor to provide estrogenic effects[45]. This reason causes isoflavone class of compounds to have an anti-osteoporosis effect marked by an increase in Runx2.

The other flavonoids, such as icariin, icaritin, and kaempferol, and the other phenolic compounds, such as phenolic cid and resveratrol (polyphenols), and the compounds from other groups, such as emodin (anthraquinone), notoginsenoside R1, ginsenoside Rg1 and – ecdysterone, also have estrogenic effects. This is because there is a hydroxyl group (OH) in these compounds, which is one of the requirements for the occurrence of estrogenic activity. Estrogenic effects will appear when it binds to estrogen receptors[46]. The binding of these compounds to the estrogen receptor in bone can cause an anti-osteoporosis effect, which is indicated by an increase in Runx2.

Increased expression of Runx2 by phytoestrogen compounds generally occurs through several signaling pathways. The phytoestrogens like glabrene, phenolic acid and kaempferol are involved in bone formation by inducing activation of the Wnt signaling pathway. Thus â-catenin penetrates the nucleus and encounters the transcription factor TCF/LEF to initiate expression of target genes, such as Runx2. The Wnt/â-catenin regulates Runx2 expression in mesenchymal cells, then controlling osteoblast differentiation and skeletal development. Activation of the Wnt signaling pathway also affects the increase in osteoblast-specific genes, such as ALP, Osx, BGP, and type I collagen to promote osteoblast differentiation and maturation[36, 47, 48, 49].

The next signaling pathway is BMP-2, which is a major growth factor that promotes the differentiation of mesenchymal cells into osteoblasts or chondroblasts[61]. The phytoestrogens resveratrol and SPI increase Runx2 expression through activation of the BMP-2 signaling pathway, cause promoting mesenchymal cell proliferation and osteoblast differentiation[38,52]. BMP-2 shows osteogenic action by activating Smad1/5/8 signaling and regulating the transcription of osteogenic genes, including distal-less homeobox 5 (Dlx5), which is a key mediator of BMP-2-induced Runx2 expression[59].

The increased expression of Runx2 was influenced by G-protein-coupled receptor 30 (GPR30) too. GPR30 is a membrane-bound ER whose role is to mediate the action of non-genomic estrogens by stimulating Cyclic adenosine 3,5-monophosphate (cAMP). The research of Khan et al[54] demonstrated that prunin stimulates osteoblast proliferation and differentiation by specifically activating GPR30, which causes an increase in cAMP levels in osteoblasts. Furthermore, activation of cAMP-dependent Erk/MAP kinase will upregulate Runx2 protein, thereby inducing osteoblast cell differentiation and bone formation.

Besides regulating osteoblast differentiation, Runx2 also plays a role in regulating the expression of several osteoblastic genes such as type I collagen, osteopontin, osteocalcin, and bone sialoprotein, by binding to OSE2[62]. Increased expression of collagen type 1 markers and osteocalcin also indicate increased

osteoblastic activity. Type I collagen acts as the main bone matrix[63]. Osteocalcin plays an important role in the process of mineralization and calcium ion homeostasis, and is often used as a marker of osteoblast differentiation[62]. Runx2 can also affect ALP marker recognition. ALP plays an important role in enhancing bone mineralization by providing phosphate as a result of hydrolysis of pyrophosphate, an inhibitor of hydroxyapatite propagation[64]. Another transcription factor that also plays a role in bone formation is osterix (Osx).

Runx2 Observation Method

Western Blot

Western blot is a biochemical technique used to identify specific proteins in complex sample mixtures. This method was developed in 1979 and combines electrophoretic screening with immunoassays for semiquantitative protein assays[65]. By this technique, it is possible to detect a single protein from a sample, but also obtain molecular weight information about that protein[66]. In the western blot method, proteins are recognized by specific antibodies as bands at certain positions on the membrane. The position is calibrated as the molecular weight of the protein in kilo Daltons (kD). If the band appears at a position on the membrane too far from the expected position of the theoretical molecular mass of the protein in question, the band is often considered a nonspecific protein[67].

The procedure for detecting Runx2 with western blot includes sample preparation, protein quantification, electrophoresis, electrical transfer, blocking, incubation of primary antibodies, washing, incubation of secondary antibodies, and detection using chemiluminescence techniques. Based on these steps, the western blot method uses an indirect method (Figure 3), because two different antibodies are used, namely primary antibodies and secondary antibodies. With the indirect method, the signal given is stronger because several secondary antibodies bind to each primary antibody[29].

Immunohistochemistry

Immunohistochemistry (IHC) is a method used to determine the expression of biomarkers in tissues[69]. This technique basically identifies tissue constituents (antigens) through antigen-antibody interactions. The target antigen/protein in the tissue is recognized by antibodies that are highly specific to that antigen[27, 70, 71]. In addition, with this technique, the distribution and localization of biomarkers or differentially expressed proteins in different parts of biological tissues can be determined[72].

The Runx2 detection procedure by immunohistochemistry method includes fixation, tissue implantation on slides, tissue cutting, deparaffinization, antigen retrieval, blocking, incubation of primary antibodies, washing, incubation of fluorescent secondary antibodies, washing, addition of chromogen substrate, washing, and detection using fluorescence microscopy. Based on these steps, the immunohistochemistry method used is an indirect method (Figure 4), because two different antibodies are used, namely primary and secondary antibodies.

Parameter Comparison

Selectivity

Selectivity is the preferred method for binding its target protein among competing sample proteins in heterogeneous mixtures[74]. The selectivity of western blot and immunohistochemistry methods for detecting Runx2 protein in bone tissue can be affected by the use of specific antibodies. However, in western blot, that protein separation also affects the selectivity of the method[75, 76].

The specific antibodies cause the target protein in the complex mixture to be found and bound selectively[74]. The selectivity of specific antibodies allows detection of target proteins in complex mixtures containing >100,000 different proteins[76]. The use of anti-Runx2 as a primary antibody to detect Runx2 protein in bone tissue is one of the factors in achieving the selectivity of western blot and immunohistochemistry methods. Anti-Runx2 will specifically bind to the Runx2 protein in bone tissue so that Runx2 can be detected. On the other hand, to increase the selectivity, the western blot and immunohistochemistry methods use blocking buffers which function to bind non-specific proteins to the membrane surface, so that the possibility of antibodies to bind the other proteins than the target protein is reduced[74, 77].

The selectivity of western blot is also affected by the separation of proteins of different sizes by gel electrophoresis, the gel commonly used is sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The presence of SDS causes the protein to become negatively charged because it binds to the SDS anion. SDS

that wraps around the polypeptide backbone causes protein denaturation. The negative charge is imparted by SDS to the polypeptide chain as long as to its length. Therefore, proteins can be separated according to their molecular weight[76]. The molecular weight of the identified protein can be determined using a known molecular weight standard[77].

The determination of the selectivity value is based on the false positive and true negative values in the detection results. False positives are described as the number of samples with positive results in the western blot or IHC test, but negative in the standard method test. Meanwhile, true negative is described as the number of samples with negative results both in the test using the standard method and with the western blot or IHC method. The selectivity value is calculated by the following formula[78-80].

$$\frac{True\ negative}{True\ negative\ +\ False\ positive} \times 100\%$$

Research by Arzouni et al[81] showed that the western blot method was more selective in detecting antibodies to indicate bacterial infection than the immunofluorescence method. The research results of Liu et al[82] that detected antibodies for infection by protozoan parasites using the western blot and indirect immunofluorescence antibody methods showed that western blot was more selective in detecting these antibodies than indirect immunofluorescence antibodies, with selectivity values of 100% and 91.4–95.8%, respectively. The results of the study from Jaskowski et al[80] showed that the western blot method was more selective in detecting antibodies to markers of autoimmune disorders than indirect immunofluorescence antibodies, with selectivity values of 100% and 94.3%, respectively. According to research by Lindenmayer et al[78], the western blot method is more selective in detecting antibodies that indicate bacterial infection than the indirect immunofluorescence antibody method, with selectivity values of 100% and 93.5%, respectively. Thus, western blot is a more selective method for detecting proteins (including Runx2) in bone tissue than immunohistochemistry.

Sensitivity

Sensitivity is described as the ability of a method to detect a certain amount of protein. Western blot is a method with very high sensitivity, and is more sensitive than the immunohistochemistry method[73]. This method can detect target proteins especially with low abundance (nano to pico gram) because of their high resolution capacity of gel electrophoresis and because of the high affinity of antibodies to their epitope[76, 83-85]. The final detection/amplification system of western blot also affects the sensitivity of the method[83, 84]. For example, the final detection method of chemiluminescence is more sensitive than fluorescence[86].

The determination of the sensitivity value is based on the true positive and false negative values in the detection results. True positive is described as the number of samples with positive results, both in the test using the standard method and with the western blot or IHC method. Meanwhile, false negative is described as the number of samples with negative results in the western blot or IHC test, but positive in the standard method test. The sensitivity value is calculated by the following formula[78–80].

$$\frac{True\ positive}{True\ positive\ +\ False\ negative} \times 100\%$$

Based on the research of Atehortúa et al[87], western blot method is more sensitive than immunohistochemistry in detecting protein markers of muscular dystrophy, with sensitivity values of 100% and 99.5%, respectively. Na et al[88] in their study also showed that the western blot method is more sensitive than immunohistochemistry in the detection of protein markers of muscular dystrophy. Arzouni et al[81] showed that the western blot method is more sensitive in detecting antibodies that indicate the presence of bacterial infection than the immunofluorescence method. Basso et al[89] showed in a study that detected antibodies for infection markers by parasitic protozoa using the western blot and indirect immunofluorescence antibody methods that western blot was more sensitive in detecting these antibodies than indirect immunofluorescence antibodies, with sensitivity values of 93.5% and 87.3%, respectively.

According to research by Lindenmayer et al[78], the western blot method is more sensitive in detecting antibodies that indicate bacterial infection than the indirect immunofluorescence antibody method, with sensitivity values of 100% and 66.7%, respectively. Based on the research of

Teysseire and Raoult[79], the western blot method is more sensitive in detecting antibodies that indicate the presence of a pathogenic infection than the immunofluorescence method, with sensitivity values of 67% and 46%, respectively. Research results from Jensenius et al[90] that detected various antigens based on antigen-antibody binding to determine the presence of infection by pathogens using western blot and indirect immunofluorescence antibody methods showed that western blot was more sensitive than indirect immunofluorescence antibody, with sensitivity values respectively being 100% and 45%. As a result, the western blot method is more sensitive than the immunohistochemistry/ immunofluorescence method.

Processing time

The processing time parameter describes how long the Runx2 observation process takes for each method. The total processing time was determined based on the time required for each step of the western blot and immunohistochemistry methods to detect Runx2 in primary data. Details of the processing time required for observations by the western blot method can be seen in Table 2, while the details of the processing time required for observations by the immunohistochemistry method can be seen in Table 3.

Based on Table 2, it can be seen that the observation of Runx2 expression by western blot method takes 25 hours and 20 minutes. Meanwhile, the observation of Runx2 expression by immunohistochemistry method took 46 hours. As a result, it can be concluded that the detection of Runx2 protein is faster by western blot method than immunohistochemistry.

Cost efficiency

The cost efficiency parameter describes how much is the cost used in the observations in each method. Based on the research of Atehortúa et al[87], the western blot method is ±57.26% more cost-effective. The immunohistochemistry method requires a fluorescence microscope for observation. This microscope is more expensive than the observation instrument used in the western blot method, namely the chemiluminescence imager. The use of a chemiluminescence imager can save up to 68.89% of costs compared to using a fluorescence microscope[96, 97]. Thus, observations using the western blot method are more cost-efficient than the immunohistochemistry method.

CONCLUSION

Western blot and immunohistochemistry methods can be used to detect Runx2 in bone tissue induced by phytoestrogens, characterized by an increase in Runx2 expression. The western blot method is more selective, sensitive, requires less cost, and is faster than the immunohistochemistry method.

Authors' Contribution

All of the authors participated actively in the research and writing of the article.

Conflict of Interest

This article's author declares that there is no conflict of interest.

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