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QUESTIONNAIRE FOR EVALUATING INFORMATION, KNOWLEDGE, AND ATTITUDES ON DONATION, STORAGE, AND APPLICATION OF INDUCED PLURIPOTENT STEM CELLS

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The discovery of Induced Pluripotent Stem Cells (*iPSCs*) opened the possibilities for reprogramming adult somatic cells back to a pluripotent state *in vitro* by inducing a forced expression of specific transcription factors. Thus, *iPSCs* might have potential application in regenerative medicine, transplantation, avoidance of tissue rejection, disease modeling, and drug testing. Because of apparent ethical issues connected with donation and derivation of biomaterials, *iPSCs* are considered as a research alternative to ethically highly disputed Embryonic Stem Cells (*ESCs*). Objective: The aim of this paper was to describe the development of a questionnaire for evaluating information, knowledge, and attitudes on donation, storage, and application of *iPSCs* (i.e., the QIPSC). We performed a prospective qualitative study based on the development, validation and reliability testing of the QIPSC. The study included 122 respondents and the final version of the QIPSC with 34 items. The reliability analysis for part of information and knowledge of respondents according to *iPSCs* was then performed with the questions included in this two-component model and obtained a Cronbach's alpha value of 0.783 and 0.870, respectively. It has been shown that the range of correct answers to questions in part of knowledge of respondents according to *iPSCs* was from 17.2-63.1%. The results of our study show that the QIPSC was a unique, reliable, and valid questionnaire for assessing the level of information, knowledge, and attitudes on donation, storage, and application of *iPSCs*.

Keyword: Questionnaires, *iPSCs*, Validity, Reliability, Validation Studies

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INTRODUCTION

The discovery of Induced Pluripotent Stem Cells (*iPSCs*) opened the possibilities for reprogramming adult somatic cells back to a pluripotent state *in vitro* by inducing a forced expression of specific transcription factors so-called *Yamanaka factors (i.e., Oct3/4, Sox2, Klf4, and c-Myc*) or using the similar set of four transcription factors so-called *Thompsons's modification of Yamnaka factors (i.e., Oct4, Sox2, Nanog, and Lin28*) (TAKAHASHI, YAMANAKA, 2006; GURDON, 2006; TAKAHASHI *et al*., 2007; YU *et al*., 2007; YAMANAKA, 2008; LOWRY *et al*., 2008; LANZA, ATALA, 2014; RANČIĆ *et al*., 2020). These type of stem cells have common features with Embryonic Stem Cells (*ESCs*) in terms of surface antigen expression, telomerase activity, gene expression, proliferation, and morphology, also could be differentiated into the cells of all three germ layers (LAKO *et al.*, 2010; RAŠČANIN *et al*., 2019; RANČIĆ *et al*., 2020).

iPSCs might have potential application in regenerative medicine (CHENG *et al*., 2016; WANG *et al*., 2019; LIU, CHEUNG, 2020), transplantation (SILVESTRO *et al*., 2020), avoidance of tissue rejection, disease modeling (ATCHISON *et al*., 2017; MCKINNEY, 2017; VAN DEN BERG *et al*., 2019), and drug testing (ESCH *et al*., 2015; CRESPO *et al*., 2017; PANG, 2020). Because of apparent ethical issues connected with donation and derivation of biomaterials, *iPSCs* are considered as a research alternative to ethically highly disputed *ESCs* (MOHAMED, 2018; RAŠČANIN *et al*., 2019). But it is very important to note that there is a risk of tumorogenesis. More research is needed to fully understand the reprogramming system and how *iPSCs* can be controlled to produce a sufficient number of cells with high quality and safety requirements for use in stem cells treatments (WAKUI *et al.*, 2017; VOLAREVIC *et al*., 2018; RAŠČANIN *et al*., 2019; ZAKRZEWSKI, 2019; RANČIĆ *et al*., 2020).

Some studies showed that the public was familiar with the terms *iPSCs*, such as Japanese public, they also support research with *iPSCs*; namely, an internet-based survey was conducted to determine public opinion about the research and development of *iPSCs* and regenerative medicine (SHINEHA *et al*., 2010; SHINEHA, 2016; ISHIHARA *et al*., 2016). Further efforts are required to overcome the difference between science and society's expectation and increasing interest in these fields (SHINEHA *et al*., 2017; SHINEHA *et al*., 2018). Other studies results illustrated a gap in education and knowledge about beneficial use of stem cell therapy (SULTAN, 2017). It should be noted that public in China significantly supported use of *iPSCs* for treating human diseases, research was based on survey, and results were correlated with participant's age, education level, and geographic region (LUO *et al*., 2016). Similar research was conducted in California through a survey that was able to determine whether students were provided enough information to make educated decisions or provide educated opinions regarding stem cell research (BLANSIT, 2017). Creating an adequate questionnaire for evaluating information, knowledge, and attitudes on donation, storage, and application of *iPSCs* would be of great practical importance in terms of examining the information and level of knowledge of health professionals, as well as examining the attitudes and beliefs of the general population about donating, storing and using *iPSCs* and analyzing the relationship of attitudes and beliefs of the general population about this with different sociodemographic parameters, as well as examining differences in attitudes and knowledge between health workers and the general population.

The aim of this paper was to develop a reliable and valid questionnaire for evaluating information, knowledge, and attitudes on donation, storage, and application of *iPSCs*.

MATERIALS AND METHODS

We performed a prospective, qualitative study based on the development, validation, and reliability testing of the QIPSC developed for assessing information, knowledge, and attitudes on donation, storage, and application of *iPSCs.*

Population

The research was conducted at the Serbian-speaking areas; responders were from several Health institutions, Primary, Secondary schools, and Colleges, as well as members of the general population on the territory of Kragujevac and Belgrade. Criteria for inclusion in the study were persons older than 18 years and ability to read and write in Serbian. The sampling was random. The independent variables were gender, age, religion, ideology, socioeconomic status, level of education, and the dependent variables were information and level of knowledge. The study surveyed a total of 122 respondents, 48 males and 74 females.

Creating the questionnaire

Questionnaire development was performed in a standardized way, using the accepted methodology for the development and validation of the questionnaire. The design of this questionnaire began with a search of bibliographic databases. Three focus groups were formed based on the recommendations used in the development of the other questionnaires for measuring knowledge. The focus groups consisted of 5 doctors, 5 medical students, and 5 people from the general population. The goal of the focus groups was to create an initial set of questions. The review ended with the final Questionnaire 1, Questionnaire 2, and Questionnaire 3. Questionnaire 1 contained 11 questions related to the socio-demographic characteristics of respondents: gender, age, level of education, socioeconomic status, religion, ideology, the way of informing about the subject of research, and does the media pays attention to scientific research. Questionnaire 2 contained 13 closed-ended questions about level of information about *iPSCs*. Scoring scale for the Questionnaire 2 included: 1 to 3 positive answers – Poorly informed; 4 to 6 positive answers – Partially informed; 7 to 9 positive answers – Well informed; 10 to 11 positive answers – Highly informed. Questionnaire 3 measures level of knowledge about *iPSCs* and contained 12 stated statements, the respondents answered whether they agreed or not. Scoring scale for the Questionnaire 3 included: 1 to 3 correct answers – Poor knowledge; 4 to 6 correct answers – Partial knowledge; 7 to 9 correct answers – Good knowledge; 10 to 12 correct answers – Excellent knowledge. Pilot testing of the questionnaire was inducted with 5 health professionals and 20 people from the general population by the same researcher to assess clarity and understanding. The results of the pilot testing were not taken into account during the processing of the data for the validation of the questionnaire. In the next step, the survey was conducted on 122 respondents (STONE, 1993; HINKIN, 1998; BOYNTON, GREENHALGH, 2004; ARTINO *et al.*, 2014; TSANG *et al.*, 2017).

Statistics

Complete statistic data analysis was performed using the IBM SPSS software package version 18.0®. Data were presented in the form of number (percentage). QIPSC reliability analysis was performed by determining Cronbach's α (CRONBACH, 1951; CRONBACH, MEEHL,

1955; DZIUBAN, SHIRKEY, 1974; BROWN, 2009; BUDAEV, 2010). Mutual correlations of items were analyzed with the help of a correlation matrix (DZIUBAN, SHIRKEY, 1974). The suitability of the results for factor analysis was examined using the Kaiser-Meyer-Olkin Measure of Sampling Adequacy and the Bartlett's Test (BUDAEV, 2010). Factors were extracted after orthogonal rotation using the "oblimin" method (BROWN, 2009).

RESULTS

Questionnaire 1 of the QIPSC shown that most of respondents had University degree (88.5%), and there were 12 respondents that had Master of Science and Doctor of Science degree. Most of the respondents were of good socioeconomic status (Appendix-Table 1). All respondents were of Serbian ethical affiliation, and most of them were of the Orthodox faith. The majority of respondents (66.4%) stated that they have liberal ideological views (Appendix-Table 2). Also, the majority of respondents in our study answered that they were informed about stem cells via the Internet (76.2%), and most respondents believe that this topic is given insufficient or very little attention in the media (Table 1).

Table 1. Manner and amount of informing respondents about stem cells

Stem cell information	
Via newspaper	7 (5.7%)
Via television	22 (18.0%)
Via internet	93 (76.2%)
Media attention regarding stem cells	
Very little	55 (45.1%)
Little	30 (24.6%)
Insufficient	36 (29.5%)
Enough	1 (0.8%)
Too much	/

Questionnaire 2 of the QIPSC refers to the respondents' attitudes about *iPSCs* based on information about them. Thirteen *iPSCs* information items were subjected to principal components analysis (PCA). Prior to conducting the analysis, the suitability of the data for factor analysis was assessed. Examination of the correlation matrix revealed many correlation coefficients greater than 0.3. The Kaiser-Meyer-Olkin Measure of Sampling Adequacy value of 0.75 indicates the adequacy of the sample, while the value of Bartlett's Test of Sphericity <0.001, is highly statistically significant, which all together indicates the factorality of the correlation matrix. After that, a PCA was conducted to determine how many phenomena were measured by the developed QIPSC related to *iPSCs* awareness. The analysis of the main components revealed the presence of five components with characteristic values over 1, which explain a total of 66.55% of the variance. A review of the bend diagram revealed the existence of a clear breakpoint behind the second component. Based on Katel's criteria, it was decided to keep two components for further research, which meant removing two questions (11, 12) from the QIPSC. The two-component solution explained a total of 41.09% of the variance, with the contribution

of the first component being 29.61% and the second 11.48%. To make these components easier to interpret, an oblimin rotation was performed. The rotated solution revealed the existence of a simple structure, with both components having quite high weights, the first component in the range of 0.31–0.75 and the second in the range of 0.35–0.57 (Table 2). There is a strong positive correlation between these two factors (r = 0.39). These results support the assumption that information issues should be viewed in two separate components, which are presented in this part of the results. After that, orthogonal rotation was performed using the Varimax method by which the factors were partially equalized in importance. However, since a very complicated division of the issue into five factors was obtained, it was decided to keep the solution with oblique rotation (i.e., oblimin rotation). Finally, the first component includes questions 1, 2, 3, 5, 6, 7, 8, 9, 10, and 11. The second component includes questions 1, 2, 4, 7, and 10. The internal consistency reliability analysis was then performed with the items included in this two-component model scores as one score and obtained a Cronbach's alpha value of 0.78. Removing any issue internal reliability does not change significantly. Questionnaire 2 finally contains 11 closed-eded questions.

Table 2. Component Matrix for iPSCs information issues - two components

Component Matrix[a]		
	Component	
	1	2
Information 5	0.750	
Information 3	0.750	
Information 6	0.724	
Information 8	0.701	
Information 7	0.668	-0.354
Information 9	0.661	
Information 13	0.489	
Information 1	0.389	0.574
Information 4		0.568
Information 10	0.475	-0.540
Information 2	0.310	0.445
Information 12		
Information 11		
Extraction Method: Principal Component Analysis.		
a. 2 components extracted.		

Questionnaire 3 of the QIPSC refers to the respondents' knowledge of *iPSCs*. Twelve items on *iPSCs* knowledge were subjected to PCA. Prior to conducting the analysis, the suitability of the data for factor analysis was assessed. The examination of the correlation matrix revealed many correlation coefficients greater than 0.3. The value of Kaiser-Meyer-Olkin Measure of Sampling Adequacy of 0.83 indicates the adequacy of the sample, while the value of

Bartlett's Test of Sphericity <0.001 or highly statistically significant, which all together indicates the factorality of the correlation matrix. After that, a factor analysis was conducted to determine how many phenomena the developed *iPSCs* knowledge questionnaire measures. The analysis of the main components revealed the presence of three components with characteristic values over 1, which explain a total of 63.89% of the variance. A review of the pass diagram revealed the existence of a clear breakpoint behind the third component. Based on Katel's criteria, it was decided to keep two components for further research. The two-component solution explained a total of 55.16% of the variance, with the contribution of the first component being 41.66% and the second 13.50%. To make these components easier to interpret, an oblimin rotation was performed. The rotated solution revealed the existence of a simple structure, with both components having quite high weights, the first component in the range of 0.52–0.77 and the second in the range of 0.31–0.67 (Table 3).

Table 3. Component Matrix for iPSCs knowledge issues - two components

Component Matrix[a]		
	Component	
	1	2
Knowledge 7	0.768	-0.307
Knowledge 6	0.718	0.398
Knowledge 12	0.669	
Knowledge 8	0.669	
Knowledge 9	0.657	
Knowledge 5	0.646	
Knowledge 11	0.632	
Knowledge 2	0.626	0.533
Knowledge 4	0.625	-0.318
Knowledge 10	0.620	
Knowledge 3	0.565	-0.516
Knowledge 1	0.516	0.674
Extraction Method: Principal Component Analysis.		
a. 2 components extracted.		

There is a strong positive correlation between these two factors (r = 0.40). These results support the assumption that knowledge issues should be viewed in two separate components that are presented in this part of the results. After that, orthogonal rotation was performed using the Varimax method, by which the factors were partly equalized in importance. However, since a very complicated division of the issue into four factors was obtained, it was decided to keep the solution with oblique rotation (i.e., oblimin rotation). Finally, the first component includes all the questions. The second component includes items 1, 2, 3, 4, 6, and 7. The internal consistency reliability analysis was then performed with the questions included in this two-component model and obtained a Cronbach's alpha value of 0.87. Removing any issue internal reliability does not

change significantly. It has been shown that the range of correct answers to questions is 17.2-63.1% (Table 4).

Table 4. Correct answers to the questions in the knowledge section

	Number of correct answers
Knowledge 1	66 (54.1%)
Knowledge 2	67 (54.9%)
Knowledge 3	73 (59.8%)
Knowledge 4	50 (41.0%)
Knowledge 5	57 (46.7%)
Knowledge 6	77 (63.1%)
Knowledge 7	56 (45.9%)
Knowledge 8	50 (41.0%)
Knowledge 9	39 (32.0%)
Knowledge 10	21 (17.2%)
Knowledge 11	66 (54.1%)
Knowledge 12	42 (34.4%)

DISCUSSION

The reliability analysis was performed with the questions included in two-component model and obtained a Cronbach's alpha value of 0.783 (Questionnaire 2) and 0.870 (Questionnaire 3) which indicates that the questionnaire is very reliable and any issue to remove internal reliability does not change significantly. The results of the study confirm that we develop a reliable and valid QIPSC for assessing and measuring the level of information, knowledge, and attitudes about donating, storing, and application of *iPSCs.* Some demographic data of respondents in Japan were similar, concerning their ages the most of them were 20-70 years old as in our study, 63.2% were male and 36.8% female, in our study 39.3% were male and 69.7% female. The majority of respondents in Japan alsow informed about these terms by newspapers or internet 73.5% (SHINEHA *et al*., 2010) as in our research. In our study it has been shown that the range of correct answers to stated statements of knowledge about *iPSCs* was 17.2-63.1%. So we can say that knowledge of respondents in our study was at a satisfactory level. A survey conducted in India showed that 53.7% of respondents were aware of the use and benefits of stem cells (SULTAN, 2017). Similar research has been conducted in China to assess the attitudes of medical workers. They found that the level of factual knowledge about stem cell science appeared to be high among their medical workers, as many as 99.1% of them had heard of stem cell research and the internet, and newspapers were the most common ways to find informations on stem cell research (LUO *et al*., 2016). Research that has been conducted in Japan shown that some demographic data of their respondents were very similar to our study (SHINEHA *et al*., 2010). Many other studies shown assessments of respondents' attitudes about stem cells (FRATI *et al*., 2014; LYE *et al*., 2015; SHINEHA, 2016; ALLUM *et al*., 2017; ALHADLAQ *et al*., 2019), some of them shown estimates respondents' information and attitudes about iPSCs (SHINEHA *et al*., 2010; ISHIHARA *et al*., 2016; MCCAUGHEY *et al*., 2016; SAWAI *et al*., 2017). A

common problem in many stem cell research studies was attitudes of respondents about stem cells as well as legal constrains. In recent years, the public has been more supportive of stem cell research. Many researchers also used a survey or questionnaire for collecting data about information and attitudes of respondents as we did, but unlike most we were more focused on our questionnaire development as reliable and valid measuring instrument for information, knowledge, and attitudes on donation, storage, and application *iPSCs*.

CONCLUSION

The issue of public opinion attitudes can greatly influence further research with *iPSCs*. Records of research with *iPSCs* in the world indicate that the crucial role in the continuation or suspension of research is played by the attitudes of the general population, and accordingly, appropriate legislation is adopted that limits or prevents further research. Therefore, it is necessary to test the knowledge and attitudes of both the general population and medical workers, and the best instrument for collecting this type of data is the questionnaire as reliable and valid measuring instrument.

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UPITNIK ZA PROCENJIVANJE INFORMISANOSTI, ZNANJA I STAVOVA O DONIRANJU, SKLADIŠTENJU I PRIMENI INDUKOVANIH PLURIPOTENTNIH MATIČNIH ĆELIJA (QIPSC)

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Izvod

Otkriće indukovanih pluripotentnih matičnih ćelija (*iPSCs*) otvorilo je mogućnosti za reprogramiranje adultnih somatskih ćelija u pluripotentno stanje *in vitro* indukovanom i forsiranom ekspresijom specifičnih transkripcionih faktora. Dakle, *iPSCs* mogu imati potencijalnu primenu u regenerativnoj medicini, transplantaciji, pri čemu bi bilo izbegnuto odbacivanje transplantiranih tkiva, modelovanju bolesti, ispitivanju lekova. Zbog očiglednih etičkih problema u vezi sa doniranjem i dobijanjem biomaterijala *iPSCs* se smatraju boljom alternativnom za istraživanje nego što su vrlo često etički osporavanje embrionalne matične ćelije (*ESCs*). Cilj ovog rada bio je da opiše razvoj upitnika za procenu informisanosti, znanja I stavova o doniranju, čuvanju i primeni *iPSCs* (tj. QIPSC). Sproveli smo prospektivnu kvalitativnu studiju zasnovanu na razvoju, validizaciji i ispitivanju pouzdanosti QIPSC. Studija je obuhvatila 122 ispitanika, a konačna verzija QIPSC, sadržala je ukupno 34 pitanja. Zatim je izvršena analiza pouzdanosti dela upitnika koji se odnosi na informisanost i znanja ispitanika o *iPSCs* sa pitanjima uključenim u ovaj dvokomponentni model i dobijena Kronbahova alfa vrednost od 0,783, odnosno 0,870. Pokazalo se da je raspon tačnih odgovora na pitanja u delu znanja ispitanika o *iPSCs* bio od 17,2-63,1%. Rezultati naše studije pokazuju da je korišćeni QIPSC bio jedinstven, pouzdan i valjan instrument za procenu nivoa informisanosti, znanja i stavova o doniranju, čuvanju i primeni *iPSCs.*

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